

Tolerance to Orthostatic Stress and Human Cardiovascular Control.

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Summary.

There are three principal factors relating to human cardiovascular (CV) control during orthostatic stress that require further examination and collectively may improve understanding of human CV control and orthostatic tolerance. The aim of this thesis was to examine further these factors, which were the effects of; repeated orthostatic stress, manipulation of resting blood pressure (BP), and stimulation of CV receptors.

In chapter 3, subjects were exposed to orthostatic stress on three occasions to examine a possible affect on orthostatic tolerance. The orthostatic tolerance of this group increased in the third exposure to orthostatic stress. To further assess this affect on orthostatic tolerance, a second group of subjects completed five daily exposures to orthostatic stress. In this study there was no residual effect of orthostatic stress on human CV control or orthostatic tolerance.

A second factor that may affect human CV control and orthostatic tolerance is the level of resting BP (chapter 4). Subjects completed isometric exercise training of the legs and of the arms, which both resulted in a reduction in resting systolic BP. There was an increase in orthostatic tolerance following leg training, but not following arm training. This suggested that there was no affect of the reduced resting BP on orthostatic tolerance, since the change in tolerance was only present after leg training. Hence, the increase in orthostatic tolerance may have been associated with a peculiarity of the leg training. However, resting BP was no longer reduced immediately prior to the post-training orthostatic stress test and therefore it is still possible that reduced resting BP at this point could affect human CV control and orthostatic tolerance.

The role of receptors in human CV control and orthostatic tolerance was also assessed. In the first study in chapter 5, carotid sinus baroreceptors were stimulated by applying suction or pressure to the neck of subjects, with simultaneous recording of forearm blood flow (FBF) and R-R interval. The results suggested that FBF was not controlled by the carotid sinus baroreceptors during changes in carotid sinus distending pressure, which also occur during orthostatic stress. Therefore, the carotid sinus baroreceptors may not be important in human CV control of peripheral blood flow during orthostatic stress, but appear to be important in modulating changes in R-R interval. Furthermore, the affect of chemoreceptor stimulation on forearm blood flow is not clear and its effect on orthostatic tolerance is not known. Chemoreceptor stimulation was induced by the inspiration of air containing 5% CO₂ (hypercapnia). During hypercapnia there was no change in FBF, but there was an increase in orthostatic tolerance. This increase in tolerance was accompanied by a greater reduction in stroke volume and a delayed onset of presyncope.

Human CV control and orthostatic tolerance do not appear to be affected 24 hours after exposure to orthostatic stress itself or by a reduction in resting BP induced by isometric exercise training. However, a small core of subjects may show increases in tolerance upon repeated exposure to orthostatic stress. Isometric leg training and chemoreceptor stimulation did affect human CV control and orthostatic tolerance, perhaps by an alteration in the normal distribution of blood during orthostatic stress. Further, established responses in FBF during orthostatic stress do not appear to be modulated by carotid sinus baroreceptors or systemic chemoreceptors.

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List of abbreviations.

BCP	Bromcresol purple.
BP	Blood pressure.
CBF	Cerebral blood flow.
CC	Calf circumference.
Cr	Creatinine.
CSI	Cumulative stress index.
CV	Cardiovascular.
CVP	Central venous pressure.
Δ	Peak value minus control value.
ΔBV	Estimated % change in blood volume.
$\Delta mmHg$	Change in internal chamber pressure.
$\Delta R-R$	Change in R-R interval.
DBP	Diastolic blood pressure.
DBP _{control}	Diastolic blood pressure prior to LBNP.
DNP	Duration of negative pressure.
ECG	Electrocardiography.
EMG	Electromyography.
ETCO ₂	End-tidal carbon dioxide.
FBF	Forearm blood flow.
FENa ⁺	Fractional excretion of sodium.
FVR	Forearm vascular resistance.
Hb	Haemoglobin.
Hct	Haematocrit.

HR	Heart rate.
HR _{control}	Heart rate prior to LBNP.
LBNP	Lower body negative pressure.
LLV	Lean leg volume.
LTI	LBNP tolerance index.
LV	Leg volume.
MAP	Mean arterial pressure.
MNP	Magnitude of negative pressure.
MSNA	Muscle sympathetic nerve activity.
mV	Millivolts.
MVC	Maximum voluntary contraction force.
N	Newton.
PA	Plasma albumin.
P _{aCO2}	Arterial partial pressure of carbon dioxide.
P _{Cr}	Plasma creatinine.
P _{CO2}	Partial pressure of carbon dioxide.
P _{ETCO2}	End-tidal partial pressure of carbon dioxide.
P _{Na+}	Plasma sodium.
PP	Pulse pressure.
\dot{Q}	Cardiac output.
RA	Room air.
SBP	Systolic blood pressure.
SBP _{control}	Systolic blood pressure prior to LBNP.
SV	Stroke volume.

TTP	Total plasma protein.
U _{Cr}	Urine creatinine.
U _{Na+}	Urine sodium.
V _E	Minute ventilation.

Chapter 1.

Introduction.

1.1 Introduction.

The maintenance of blood pressure within the human cardiovascular system is critical for effective cardiovascular function, particularly when exposed to gravitational stress during upright posture. However, human cardiovascular (CV) control of blood pressure (BP) homeostasis is not fully understood. Furthermore, human CV responses in the regulation of BP homeostasis during upright posture are complex. The complexity of these responses may be associated with the apparent poor design of the human CV system for sustaining upright posture, with the superior location of the heart with respect to the majority of the distensible (compliant) blood vessels of the vascular system (Rowell, 1993). The pressure that blood exerts has a hydrostatic element that is dependant on gravitational forces and is principally affected by the posture of the body (Amberson, 1943). During upright posture, hydrostatic pressure at the feet is approximately 100mmHg higher than at the point of hydrostatic indifference just below heart level (Folkow and Neil, 1971; Blomqvist and Stone, 1983). The distribution of blood within the CV system is dependant on the diameter of the blood vessels and the direction of the gravitational force, which in turn influence the volume of blood pumped by the heart (Blomqvist and Stone, 1983), thus effecting the regulation of BP homeostasis.

Upright posture (orthostasis) in humans can lead to a loss of consciousness (syncope) and this phenomenon is particularly common during motionless orthostasis and it is associated with inadequate cardiovascular control of BP and blood distribution. However, the human CV responses, in regulating BP homeostasis, to

orthostasis are normally adequate and perhaps only become ineffective when upright posture is passive and sustained for a prolonged period. The CV responses are characterised by vasoconstriction of the peripheral vasculature and increases in heart rate (Amberson, 1943; Stevens, 1966; Sather et al, 1986; Fortney et al, 1992).

Whilst it is beyond the scope of this thesis to examine every aspect of human CV control during stresses similar to orthostasis (orthostatic stress), the aim of the proceeding studies (chapters 3, 4 and 5) was to provide deeper understanding of three principal factors that may affect human CV control, in particular during orthostatic stress, that are not fully understood. Within the concept of tolerance to orthostatic stress and human CV control, this thesis will have three components which will focus upon the effects of: orthostatic stress itself, manipulation of resting BP, and CV receptor stimulation on tolerance to orthostatic stress.

1.2 Disturbances in blood distribution and blood volume during orthostatic stress.

During orthostasis the primary haemodynamic event is a translocation of blood from the central circulation to the large dependant vessels in the lower extremity vasculature (Amberson, 1943; Fortney et al, 1992; Convertino, 1993). Estimates of normal blood distribution within the human CV system suggest that the venous system contains approximately 85% of the total blood volume, with the remaining 15% contained within the arterial system (Gauer and Henry, 1976). The review by Blomqvist and Stone (1983) provided further detail concerning normal distribution of

blood within the human CV system. They suggested that approximately 70% of total blood volume is contained within systemic veins, 15% in the heart and lungs, 10% in systemic arteries and 5% in the capillary beds. These estimates of the distribution of total blood volume were presumably made during supine rest (i.e. minimal orthostatic stress) although there was no description of posture.

Despite the majority of total blood volume being situated in systemic veins, there appears to be further capacity for blood to pool. The effect that orthostatic stress has on the distribution of blood volume is primarily dependant on the compliance of the veins, mainly in the lower extremity. The volume of blood pooled in the lower extremity during orthostasis amounts to approximately 500mL in the veins of the legs (Gauer and Thron, 1965) and 300mL in the veins of the pelvic region (Wolthuis et al, 1974). A progressive increase in transit time for venous return occurs and results in a reduction in central blood volume (central hypovolaemia; Rowell, 1993). Furthermore, the volume of blood sequestered into the lower extremity increases at a given severity of orthostatic stress due to the vasoelastic properties of veins, described as delayed compliance (Rowell, 1993). The volume of blood pooled during a given level of orthostatic stress has been shown to reduce following application of external pressure (Halliwill et al, 1998) or an increase in lower limb muscle tension, that is known to improve the return of blood to the heart (venous return) via the 'muscle pump' (Amberson, 1943; Coles et al, 1956; Smith et al, 1987).

The central hypovolaemia that occurs during lower extremity blood pooling is further compounded by an increase in capillary filtration leading to a reduction in the plasma

component of blood into the interstitium (Fawcett and Wynn, 1960; Hagan et al, 1978; Hilderbrandt et al, 1993; Brown and Hainsworth, 1999). This loss of plasma volume has been demonstrated by increases in plasma protein concentration and haemoconcentration (Noddeland et al, 1981; Lundvall and Bjerkhoel, 1995).

Increased capillary filtration under orthostatic stress leads to an expansion of interstitial volume (oedema), which results to an increase in tissue fluid pressure and a reduction in capillary transmural pressure (Rowell ,1993). Furthermore, the rate of capillary filtrate re-absorption has been shown to be slower than the rate of filtration during orthostatic stress (Hiderbrandt et al, 1993). Accumulation of oedema, perhaps following repeated exposure to orthostatic stress may reduce venous transmural pressure and limit the extent of blood pooling during orthostatic stress in a similar way to greater external pressure (Halliwill et al, 1998) or increases in muscle tension (Amberson, 1943; Coles et al, 1956; Smith et al, 1987), which has been suggested previously (Hilton et al, 1988), however there is little information regarding this possibility. If cumulative oedema occurs following repeated exposure to orthostatic stress, the kidney may play an important role in maintaining blood volume or more specifically plasma volume under these conditions by manipulating the excretion of sodium in the urine.

In addition to the losses in plasma volume during orthostasis (Fawcett and Wynn, 1960; Hagan et al, 1978; Hilderbrandt et al, 1993; Brown and Hainsworth, 1999), central hypovolaemia results in reduced right atrial pressure (Murray et al, 1968; Johnson et al, 1974). Changes in central or total blood volume (possibly resulting

from reductions in plasma volume) appears to affect stretch receptors in the atria, which in turn affect the volume of water excreted by the kidney (Gauer and Henry, 1976). Indeed reductions in central blood volume have been shown to induce reductions in sodium (Na^+) excretion by the kidney (Gilbert et al, 1966; Mauran et al, 1998), and increases in central blood volume have been shown to increase Na^+ excretion (Boening et al, 1972). However, it is not known if there are effects of repeated exposure to orthostatic stress on Na^+ excretion of sodium and therefore water by the kidney.

In addition to the possibility that increased tissue fluid pressure induced by oedema formation could reduce lower extremity blood vessel transmural pressure and therefore blood pooling, a simple expansion of total blood volume may attenuate the degree of central hypovolaemia during orthostatic stress. Indeed, it has been shown previously that an increase in dietary salt, induces an expansion of plasma volume and an increase in tolerance to orthostatic stress (El- Sayed and Hainsworth, 1996; Mtinangi and Hainsworth, 1998). Therefore, these changes in blood volume and blood distribution suggests that exposure to orthostatic stress itself may induce changes in the human CV system that affect human CV control during subsequent exposure to orthostatic stress. However, this possible effect of orthostatic stress on changes on blood volume has not been examined experimentally.

1.3 Human cardiovascular responses to disturbances in central blood volume during orthostatic stress.

The principal human CV responses to disturbances in total and central blood volume during orthostatic stress include, increases in heart rate (HR) and vasoconstriction of the periphery in response to reduced central venous pressure and cardiac filling pressure (Stevens, 1966; Klein et al, 1969; Sather et al, 1986; Wahbha et al, 1989; Rowell, 1993). This reduction in cardiac filling pressure leads to a decrease in stroke volume (SV) and cardiac output (\dot{Q} ; Stevens, 1966; Blomqvist and Stone, 1983; Al-Shamma and Hainsworth, 1985; Fortney et al, 1992). The reduction in \dot{Q} occurs since the increase in HR is inadequate and cannot be elevated sufficiently to maintain \dot{Q} , possibly due to cardiac filling pressure imposing limitations (Rowell, 1993). The reductions in SV and \dot{Q} approximate 30% and 20% respectively (Bevegård, 1960) during orthostasis. Therefore, responses in HR during orthostatic stress appear to be dependant on the volume of blood lost from the central circulation, which is pooled in the lower extremity. However, there also seems to be an upper limit to this response, which is dependant on cardiac filling pressure.

Vasoconstriction of the peripheral blood vessels and the splanchnic region are established responses to orthostatic stress (Stevens, 1966; Johnson et al, 1974; Wolthuis et al, 1975; Sather et al, 1986; Fortney et al, 1992). This is associated with increases in vascular resistance and is essential in avoiding abrupt reductions in BP upon the assumption of upright posture. Reductions in blood flow to the splanchnic region *per se* (Culbertson et al, 1951), kidneys (Hesse et al, 1978; Ring-Larson et al,

1982), skeletal muscle (Brigden et al, 1950) and skin (Amberson, 1943; Johnson et al, 1974b) have been reported previously. However, the most common method by which changes in peripheral vasomotor tone, during orthostatic stress, are estimated is by the use of forearm occlusion plethysmograph (Whitney, 1953). The mercury-in-silastic strain gauges used in this technique have also been used previously to measure changes in calf circumference, indicating possible differences in the magnitude of blood pooling in the lower limbs during orthostatic stress (Fortney et al, 1992). However, it should be noted that this method does not adequately represent changes in the magnitude of blood pooling in the lower extremity *per se*, during orthostatic stress (Wolthuis et al, 1975).

Thus, a reduction in \dot{Q} , despite increases in HR and peripheral vasoconstriction, results in a progressive decrease in systolic blood pressure (SBP) as the pooling of blood in the lower extremity gradually increases. Mosqueda-Garcia et al (2000) suggested that the reductions in SBP during orthostatic stress are detected by baroreceptors, and result in a reflex increase in sympathetic activity, thus providing a stimulus for the increases in HR and peripheral vasoconstriction. Conversely, there is a slight increase in diastolic blood pressure (DBP) induced by increases in peripheral vascular resistance that are proportional to the decrease in \dot{Q} (Blomqvist and Stone, 1983). Changes in peripheral vascular resistance have been inferred from estimates of changes in forearm blood flow (FBF; Stevens, 1966; Lightfoot et al, 1991). Therefore, mean arterial pressure (MAP) is maintained reasonably well (Stevens, 1966) and hence there is a reduction in pulse pressure (PP; Eckberg and Sleight, 1992). Furthermore, the reduction in SBP during orthostatic stress can reach

an apparent 'critical level' of less than 80mmHg, which has been used as an indication of impending syncope (Murray et al, 1968). This quantification for a lower limit in SBP prior to syncope may illustrate the importance of resting SBP immediately before orthostatic stress since this would affect the range through which SBP could reduce before reaching 80mmHg. Indeed, the study of Stevens (1966) suggested that lower resting BP may have been associated with lower orthostatic tolerance. However, there is a paucity of data regarding a possible link between resting SBP and orthostatic tolerance. Further detail regarding the potential importance in resting BP in human CV control during orthostatic stress is given in chapter 4, section 4.1.

1.4 The role of 'receptors' in human cardiovascular responses to disturbances in central blood volume during orthostatic stress.

The initial stimulus for vasoconstriction during orthostatic stress has been reported to be neurally mediated by blood vessel wall stretch sensitive baroreceptor reflexes (Rowell, 1993). It has been suggested previously that vasoconstriction in the forearm during low levels of orthostatic stress is mediated by the 'cardiopulmonary baroreflex' (Abboud et al, 1979; Johnson et al, 1974; Lightfoot et al, 1989; Mack et al, 1993). However, the conceptual interpretation of baroreceptors in the right heart and pulmonary vasculature as a homogenous group of receptors that produce collective reflex responses has been challenged (Hainsworth, 1990). Indeed, isolated stimulation of baroreceptors in the pulmonary vein-atrial junction and right ventricle of the dog produced CV responses that opposed the responses usually attributed to cardiopulmonary baroreceptor unloading during orthostatic stress (Carswell et al,

1970; Crisp et al, 1988). Moreover, reflex changes in vascular resistance have been reported following the stimulation of the carotid sinus baroreceptors (Wallin et al, 1975; Lindblad et al, 1982; Ebert, 1982; Victor and Mark, 1985; Duprez et al, 1987). Therefore, current understanding regarding baroreceptor control of the peripheral vasculature during orthostatic stress requires further attention. This topic is introduced in further detail in chapter 5, section 5.1.1.

Chemoreceptors in the carotid body, aortic arch and the medulla oblongata are sensitive to changes in blood oxygen and carbon dioxide concentrations, and blood pH. However, there is little information regarding the role of chemoreceptors in human CV control during orthostatic stress. Previous reports concerning the regulation of peripheral vascular resistance during hypercapnic acidosis have produced inconsistent results (Kontos et al, 1968a; Kontos et al, 1968b; De Burgh Daly et al, 1965; Rothe et al, 1990; Daugherty et al, 1967; Walker et al, 1990). However, there remains a possibility that chemoreceptors, when stimulated could have important effects on the human CV responses to orthostatic stress. A more detailed description of the potential influence of chemoreceptor activation on responses to orthostatic stress is given in chapter 5, section 5.2.1.

1.5 Human cardiovascular responses immediately prior to syncope during orthostatic stress.

As described in section 1.3, human CV responses to orthostatic stress manifest as a reduction in SV, inadequate increases in HR thus leading to a reduction in \dot{Q} . The decrease in \dot{Q} results in a reduction in SBP, despite increases in peripheral vascular resistance which increases DBP slightly.

However, if orthostatic stress remains unchanged or is exaggerated and the muscle pump is inactive, acute bradycardia and vasodilatation occur. This 'depressor' reflex presents as presyncopal signs and symptoms (Murray et al, 1968; chapter 2), a point considered to indicate orthostatic tolerance, and eventual loss of consciousness (vasovagal syncope; van Lieshout et al, 1991). Furthermore, there appears to be a paradoxical relationship between cardiovascular responses and orthostatic tolerance. Wahbha et al (1989) concluded that either abnormally small or exaggerated cardiovascular reflex responses during a hypovolaemic orthostatic stress could predispose an individual to reduced orthostatic tolerance.

The mechanisms associated with the vasovagal response are unclear (Eckberg and Sleight, 1992; Mosqueda-Garcia et al, 2000). However, abrupt withdrawal of muscle sympathetic nerve activity and a lengthening of R-R interval have been reported at pre-syncope (Wallin and Sundlöf, 1982; van Lieshout et al, 1991). Furthermore, hyperventilation with accompanying reductions in end-tidal P_{CO_2} have also been observed at pre-syncope (Morgan et al, 1997; Imms, 2000). Since cerebral blood

flow is at least partly regulated by PCO_2 in blood, a reduction in PCO_2 may have resulted in cerebral hypoperfusion, which has been suggested to be a critical element in the inducement of pre-syncope (Zhang et al, 1997; Sung et al, 2000). It is therefore possible that increases in PCO_2 may have the opposite effect on cerebral perfusion during orthostatic stress, which may suggest that chemoreceptor reflexes are important during changes in cerebral blood flow at impending syncope, as discussed in chapter 5, section 5.2.

1.6 Techniques used to study orthostatic tolerance.

There are a number of techniques that can be used to induce a translocation of blood from the central circulation into the capacitance vessels of the lower extremity. Passive standing (Amberson, 1943), head-up tilt (Graybiel and McFarland, 1941) and lower body negative pressure (Brown et al, 1965) have been used to study the cardiovascular events leading to the onset of syncope.

Passive standing simply involves standing motionless, and induces a pooling of blood in the lower extremity until the onset of presyncopal symptoms or vasovagal syncope. It is still used on occasion as a simple orthostatic challenge (Lee et al, 1999; Moore et al, 2000). Passive standing should be considered as a 'real' and not a 'simulated' orthostatic stress (orthostasis), since there is a column of blood along the longitudinal axis of the body with changes in hydrostatic pressures that are proportional to the height of the column (Blomqvist and Stone, 1983; Rowell, 1993). However, this

method does not allow any control regarding the degree of orthostatic stress applied to the subject.

During head-up tilt, subjects are placed supine on a flat surface (tilt table) before being tilted. As in passive standing, there is a hydrostatic column of blood along the longitudinal axis of the body and is an improvement on passive standing since control of lower limb muscle tension is enhanced, as the subject contact with the ground can be avoided. The possible effects of changes in muscle tension were described in section 1.2. Head-up tilt can be well-controlled and is still used as a valid orthostatic stress (Shoemaker et al, 2001). However, tilting lacks fine control of the degree of stress applied to subjects. In addition, the movement of the subject involved in tilting may make measures, such as FBF using forearm occlusion plethysmography, technically challenging. Furthermore, tilt-table tests do not often present sufficient cardiovascular stress to elicit pre-syncopal symptoms. Wahbha et al (1989) reported that only 19% of 67 patients with histories suggestive of orthostatic intolerance presented pre-syncopal symptoms during 1 hour of 60° head-up tilt. This presents difficulty when studying factors affecting orthostatic tolerance. Some authors have used negative pressure applied to the lower body, in combination with head-up tilt (Newberry et al, 1970; El-Bedawi and Hainsworth, 1994; Coats and Imms, 1999). This development of the tilt table technique to exaggerate the cardiovascular stress has increased the incidence of pre-syncope (El-Bedawi and Hainsworth, 1994). Nevertheless, these authors have not reported pre-syncope in all subjects during their orthostatic stress tests.

The application of sub-atmospheric pressure to human subjects has been used to assess various vascular and cardiovascular responses for many years. Early studies were limited to exposing single limbs to negative pressure (Coles et al, 1956). Lower body negative pressure (LBNP; application of sub-atmospheric pressure to the body below the iliac crests) was first used by Brown et al (1965) to assess the circulatory responses to blood shifts during this simulated orthostatic stress. In LBNP testing, a subject's legs are placed inside an airtight box and a seal is formed at the level of the iliac crests. Internal chamber pressure is then reduced either progressively or in a single step to simulate an orthostatic stress on the human CV system. Unlike passive standing or tilt-table testing, the cardiovascular stress during LBNP can be finely controlled and rapidly reversed providing a safe central hypovolaemic challenge (Lightfoot and Tsintgaris, 1995; further details of LBNP test procedures are given in Chapter 2).

LBNP has become a common technique in studies of cardiovascular control of blood pressure and distribution, and has been reported many times previously over the past three decades (e.g. Stegemann et al, 1974; Luft et al, 1976; Convertino et al, 1986; Smith and Raven, 1986; Frey et al, 1987; Lightfoot et al, 1989; Fortney et al, 1989; Stevens et al, 1992; Convertino, 1993; Lightfoot et al, 1994; Savard and Stonehouse, 1995; Franke et al, 1996; Ioudina and Franke, 1998; Uusitalo et al, 1998; Zhang et al, 1999). However, LBNP has some important characteristics that separate it from other orthostatic stresses. During LBNP it is unlikely that there is a continuous hydrostatic column of blood along the longitudinal axis of the body and the blood distribution pattern is perhaps a stepwise one where transmural pressures in either

the upper or lower body are uniform (Blomqvist and Stone, 1983; Rowell, 1993). Nevertheless, HR and BP changes at -50mmHg of LBNP have been shown to be similar to those responses during 70° head-up tilt (Wolthuis et al, 1974). However, the value of using LBNP becomes apparent when studying factors affecting tolerance to orthostatic stress, since it is possible to induce presyncope in all subjects (Stevens and Lamb, 1965). Therefore, LBNP was used in all the proceeding studies (excluding 5.1) in this thesis as an orthostatic stress since when assessing factors effecting tolerance it is essential to induce presyncopal signs and symptoms.

1.7 Study aims.

Human CV responses and adaptations to orthostatic stress require further work before being full understood. This thesis will focus on three important elements of human CV responses and adaptations to orthostatic stress including the effects of repeated exposure to orthostatic stress on tolerance, the effect of resting BP manipulation on tolerance to orthostatic stress and changes in the level of human CV receptor stimulation on human CV responses associated with tolerance to orthostatic stress. The aim of studying these elements of human CV control of blood pressure homeostasis is to improve understanding of human CV control during orthostatic stress to the onset of pre-syncope.

Lower body negative pressure has been used many times previously as a simulation of orthostatic stress. However, there has been only limited development of this technique. For example little is known about the reproducibility of human CV

responses to repeated exposure to orthostatic stress. Previous reports that have considered reproducibility of tolerance to orthostatic stress have shown conflicting results (see 3.1).

The principal challenge to the human CV system during orthostatic stress is to maintain BP. However, there is a lack of understanding with regard to the effect of resting BP manipulation. Lower resting BP has been suggested to be a possible cause of low orthostatic tolerance. However, this possible link between resting BP and orthostatic tolerance has not been examined previously following manipulations in resting BP.

There is confusion regarding the contribution of different human CV receptor populations on responses to orthostatic stress or their impact on orthostatic tolerance. There have been conflicting reports regarding the effect of changes in human carotid sinus baroreceptor pressure stimulus on the peripheral vasculature (see 5.1 for further detail). Furthermore, little is known about the effect of chemoreceptor stimulation on tolerance to orthostatic stress.

Therefore the aims of the present studies were to assess;

1. The effects of the disturbances in central and/or total blood volume on human CV responses to repeated exposures to LBNP (orthostatic stress). Since this research tool was custom made it was essential that the performance characteristics of subjects tested in the chamber and the effectiveness of an appropriate experimental

protocol are initially assessed prior to further research projects. Further, to examine the role of the kidney in possible changes in blood or plasma volume during repeated exposure to orthostatic stress.

2. The effects of manipulation of resting BP on human CV responses to orthostatic stress. Currently, there are no published reports that detail the effects of reductions in resting BP on tolerance to orthostatic stress.

3. The affect of non-invasive stimulation of arterial baroreceptors and systemic chemoreceptors on human CV response during orthostatic stress.

Chapter 2

Methods.

This chapter details the general methods used in this thesis which include, the construction and procedures for the operation of the LBNP chamber, the measurement and recording of electrocardiography, electromyography and blood pressure, the collection, storage and biochemical analysis of blood and urine samples, assessment of leg volume, maximum voluntary contraction force and isometric exercise, forearm blood flow and calf circumference, application of suction and pressure applied to the neck, analysis of respiratory gas and volume, and estimation of stroke volume and cardiac output.

2.1 LBNP chamber.

The LBNP chamber (figure 2.1) was constructed by the author and the main structure consisted of a wooden base onto which a sealed wooden box was erected. Negative internal chamber pressure was generated by one or two electric vacuum pumps and regulated by a system of valves. Internal chamber pressure was monitored using a digital barometer. An LBNP chamber must allow for the legs of subjects of varying stature to be inserted into the sealed box whilst maintaining a seal at the level of the iliac crests. In addition, support for the upper body, head and arms must be provided to allow the subject to remain supine at all times. The author is grateful for the help and guidance of Professor J.T. Lightfoot (University of North Carolina Charlotte, USA) during the design phase of this part of the project.

2.2 LBNP chamber construction.

The base of the LBNP chamber, which formed the internal floor of the chamber and upper body support for subjects, consisted of six legs (68 x 68 x 710mm of wood) in a rectangular configuration stabilised at the top and bottom by lengths of wood (140 x 20mm and 28 x 18mm respectively). The top of the base was formed by a single sheet of plywood (2450 x 610 x 19mm). On this base the chamber was supported and subjects adopted the supine position (figure 2.1). The height of the base was important as it allowed for better access to the subject for instrumentation and both the subject and seated experimenter were able to perform testing at approximately the same eye level for ease of communication.

The top of the base formed a platform for a number of structures. At one end (730 x 610 x 100mm) foam padding offered a comfortable support for subjects' upper body and head. Inside the chamber a bicycle saddle was mounted vertically using a specially fabricated steel bracket (figure 2.2). The purpose of the saddle was to stabilise the subject during LBNP, which aids the maintenance of the seal throughout the test. LBNP generated a mild force, pulling the subject slightly into the chamber. Any such movement by the subject may have disrupted the seal and partially restored internal chamber pressure with respect to ambient pressure.

The walls of the chamber were also bolted to this platform. Two walls (1650 x 830 x 19mm plywood) were positioned longitudinally along the length of the platform from the effective position of subjects' iliac crests, to the end of the platform (opposite to

that of the foam padding). In addition, two walls (610 x 830 x 19mm plywood) formed the ends of the chamber with a single sheet (1650 x 610 x 19mm) of plywood providing a top (figure 2.1). In one of the chamber ends (opposite the subject's head) a 390 mm diameter hole was cut to allow access to subjects' right calf for placement of a mercury-in-silastic strain gauge (see 2.17). During tests this 390 mm diameter hole was covered by a 470x13 mm diameter sheet of plywood, edged with draft excluder and held in position by six steel coach bolts and wing nuts (figure 5.1). All walls and the top of the chamber were bolted together using steel coach bolts and were braced with steel reinforcement (Dexion; figure 2.2). To minimise stress on the chamber walls during negative pressure exposures, three bulkheads were positioned between the sidewalls and the top of the chamber (figure 2.2). All wall joints and retaining bolts were sealed using a silicone sealant (Dow-Corning). Additional foam padding was also provided inside the chamber to maximise subject comfort during testing (figure 2.2).

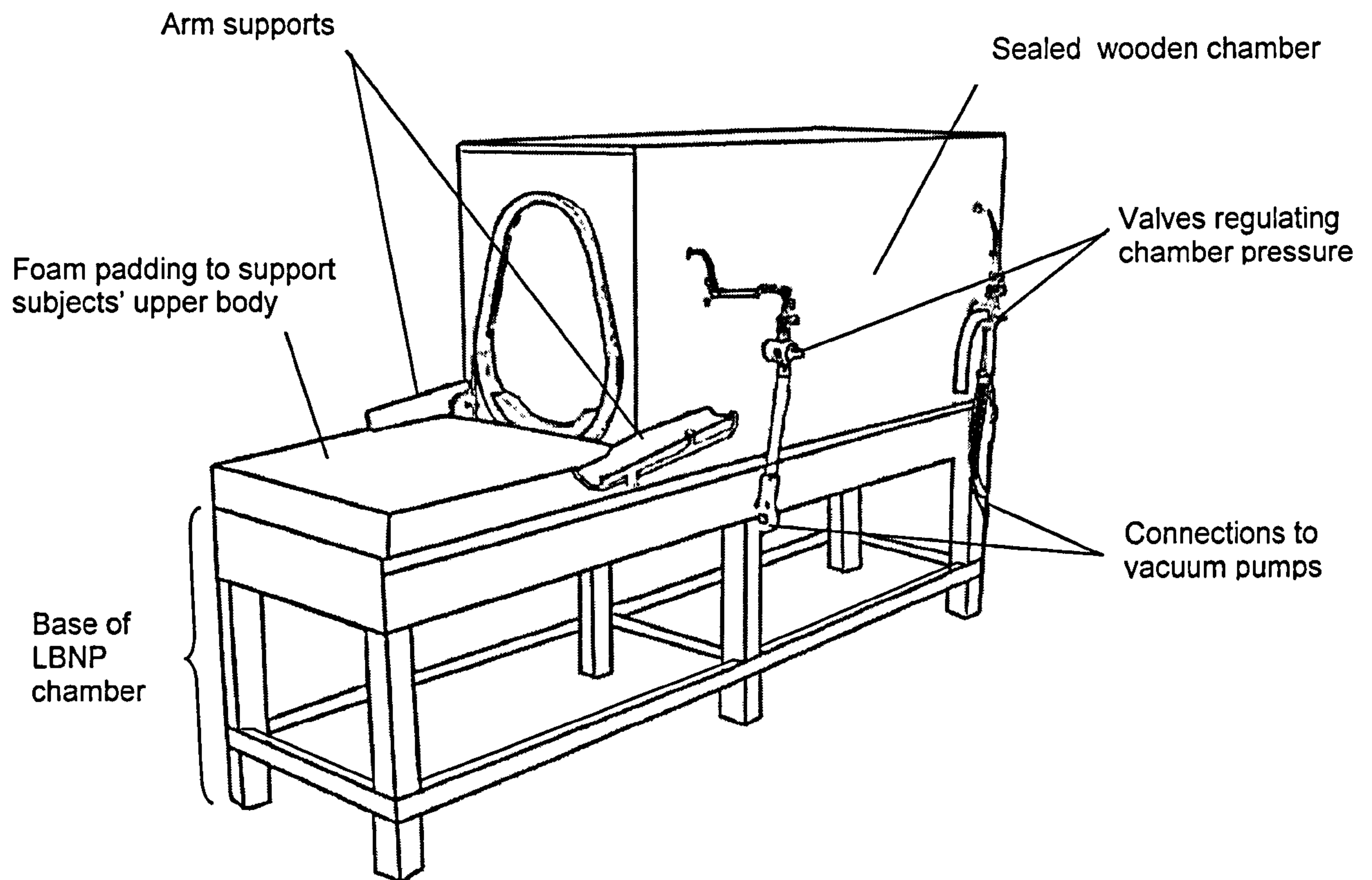


Figure 2.1 Lower body negative pressure (LBNP) chamber.

2.3 Chamber aperture and subject seal.

The aperture of the chamber allowed subjects' to place their lower extremity inside the chamber and also provided an important structure to form the seal (figure 2.2). It was necessary for the aperture to be large enough to allow subjects to enter the chamber with a degree of knee joint flexion. However, once in the supine position there was a sizeable opening superior to the subject that needed to be accounted for when preparing a suitable seal at the level of subjects' iliac crests. The closure of this opening was achieved with a custom built, adjustable 'baffle' board. This device also allowed for the opening to be closed in extremely close proximity to the subject, aiding the effectiveness of the seal. An additional strip of foam padding (430 x 45 x

35) prevented the potential loss of chamber negative pressure was positioned at the bottom of the aperture which made contact with the subject's lower back (figure 2.2).

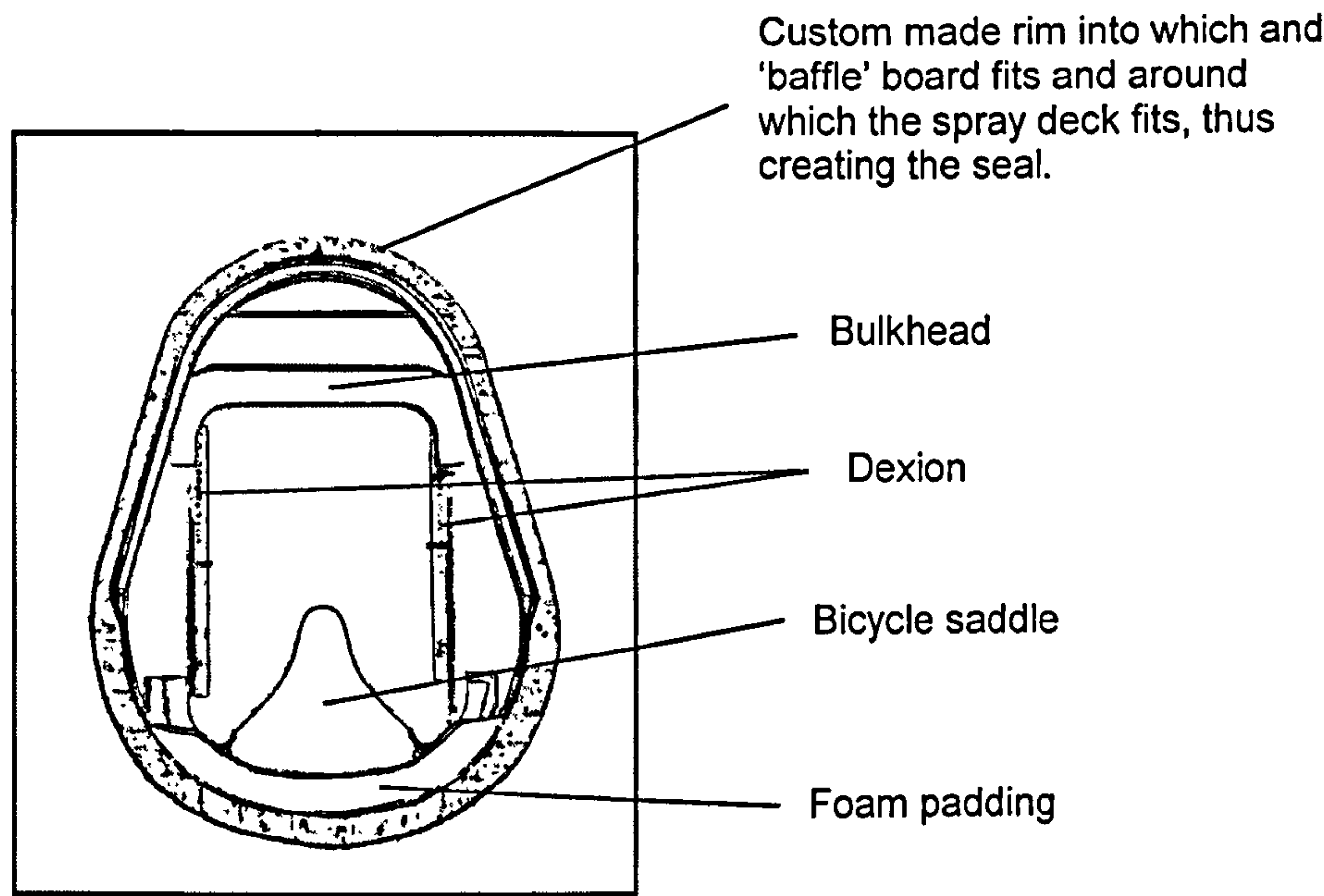


Figure 2.2 LBNP chamber aperture.

A major component of the seal was a kayak 'spray deck' (Yak, Crewsaver, UK). A spray deck of appropriate size was positioned on the subject with the body of the spray deck reversed so that the upper most contact between the subject and the spray deck was at the level of the iliac crests (figure 2.3). To improve the close contact between subject and spray deck, two small bicycle inner tubes were then placed around the body of the spray deck, slightly below the iliac crests (figure 2.3). These inner tubes could also be inflated to close any remaining gaps between subjects and the 'baffle' board or LBNP chamber aperture. The final procedure in creating an effective seal around subjects' iliac crests was to fit the outer edge of the 'spray deck' to a specially fabricated rim that encompassed the chamber aperture (figure 2.2).

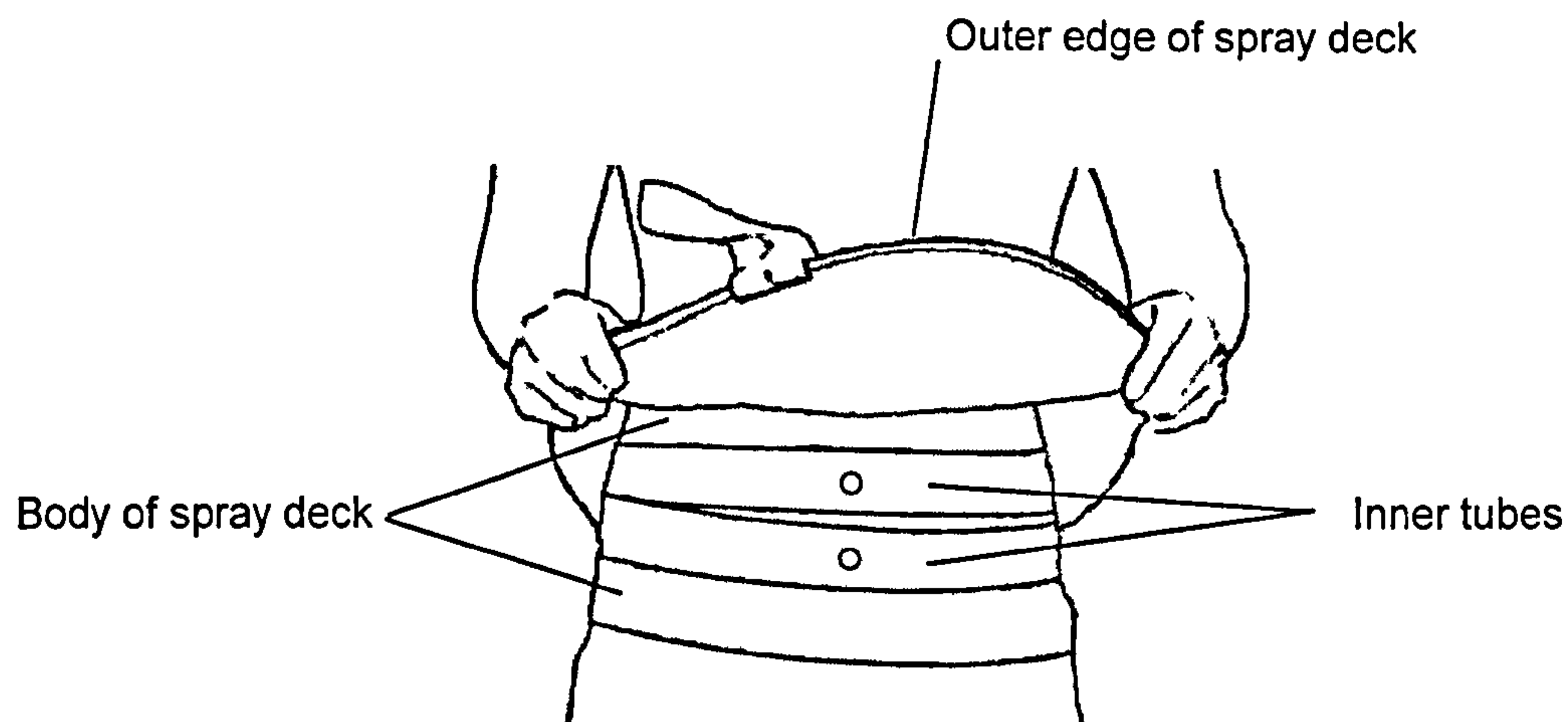


Figure 2.3 Showing spray deck and inner tubes.

2.3 Regulation of internal chamber pressure.

To simulate orthostatic stress, internal chamber pressure must be reduced with respect to atmospheric pressure. Two electric vacuum pumps (industrial vacuum cleaners, 1000 watts each) were each connected to the chamber via a sealed air tube and drew air directly from the chamber interior (figure 2.1). The sealed tubes were each interrupted by one valve (Hans Rudolph, USA), which allowed for the magnitude of suction to be finely regulated (figure 2.1).

Internal chamber pressure was measured using a digital barometer (Greisinger Electronic, Regenstauf, Germany). This digital barometer was verified against a mercury manometer (Griffin, UK) at all internal chamber pressures that were expected to be used during the present studies (i.e. -20 to -100mmHg ; figure 2.4). However, the digital barometer measured pressure in millibars (mbars) and the

preferable units for LBNP chamber pressure was mmHg for comparison with previous literature (Sáther et al, 1986; Fortney et al, 1992; El-Bedawi and Hainsworth, 1994). In addition, it was useful to record internal chamber pressure in order to calculate the LBNP tolerance index (LTI; Lightfoot and Tsintgaris, 1995) to quantify subject performance, which is described in detail in chapter 3. Therefore, an analogue output from this device was connected via a custom-made analogue cable to an analogue to digital converter (PowerLab 8sp, ADInstruments, UK) and software driven units conversion (mbars to mmHg) (Chart v 4.0.1, ADInstruments, UK) was performed with simultaneous recording of internal chamber pressure.

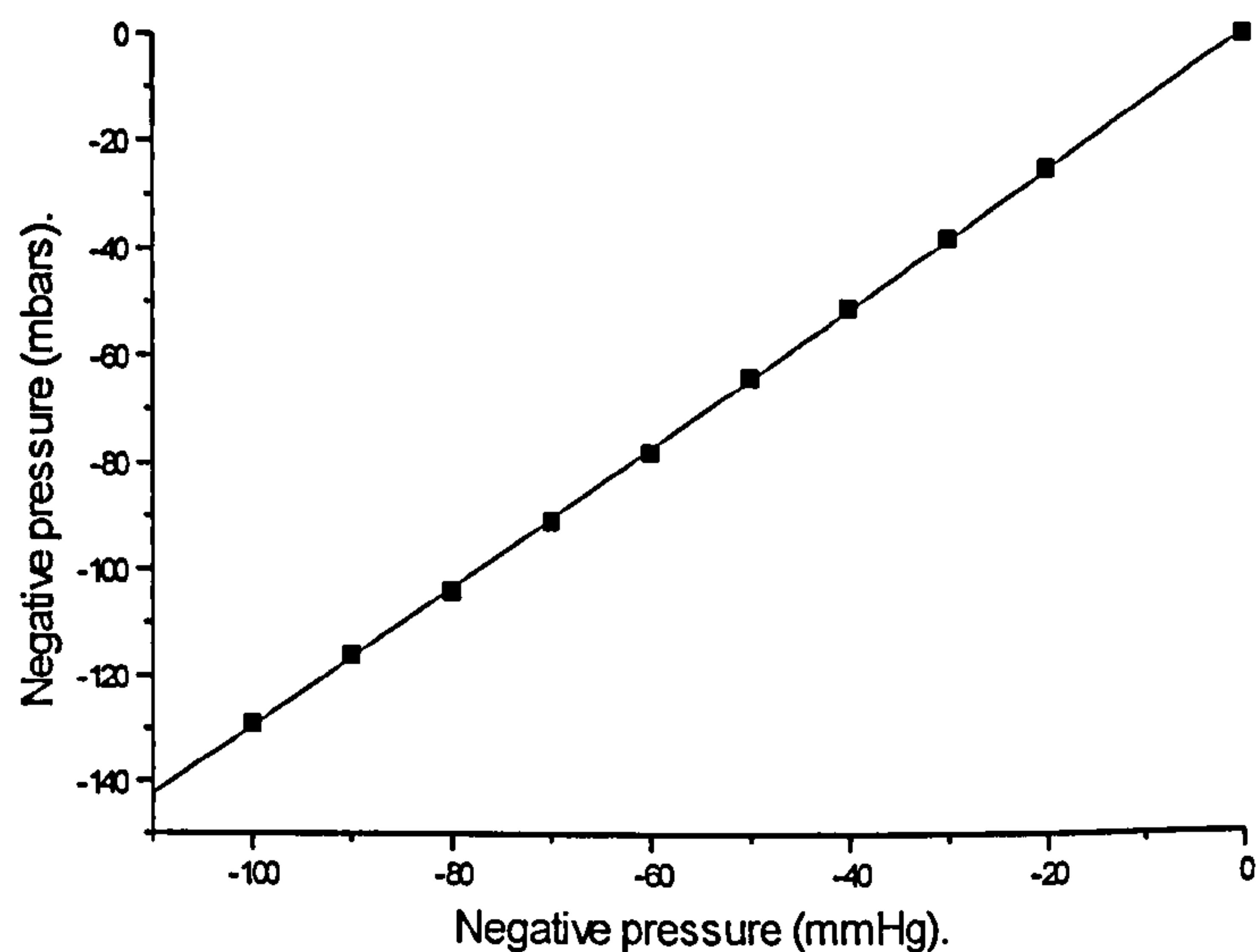


Figure 2.4 Relationship between changes in internal chamber pressure measured using a mercury manometer (abscissa) with corresponding changes in internal chamber pressure measured using the digital barometer (ordinate; $r = 0.99$; $P < 0.001$).

2.4 Pre-LBNP test screening.

Prior to all LBNP test procedures all subjects were required to complete a 'pre-test medical questionnaire' in accordance with the Physiology of Exercise laboratories, De Montfort University, Bedford pre-test procedures. Following a verbal description of all test procedures subjects were also required to provide written informed consent to participate.

2.5 Procedures for orthostatic stress testing.

Prior to the first pre-syncopal symptom limited LBNP test (orthostatic stress test), all subjects completed an initial orientation LBNP exposure to -40mmHg . A period of at least 72 hours separated any repeated LBNP test, except in studies where responses to daily exposure to LBNP were being studied. All orthostatic stress tests were conducted at an ambient temperature of $21 \pm 1^\circ\text{C}$ (except when measures of forearm blood flow were made when the laboratory temperature was maintained at $24 \pm 1^\circ\text{C}$) and each subject was tested at the same time of day ($\pm 1\text{hour}$) to account for possible circadian variation in human CV responses to orthostatic stress (Aschoff and Aschoff, 1969; Wolthuis et al, 1975). All subjects reported for orthostatic stress testing at least 12 hours post-exercise and at least 2 hours post-prandial.

Subjects were first sealed in the LBNP chamber, as described above, and remained in a supine resting state for a 5-minute control period. Chamber pressure was then reduced by 20mmHg for 3-minutes. Subsequent reductions in pressure of 10mmHg

were then made every 3-minutes until the onset of one or more pre-syncopal signs or symptoms, at which point the test was terminated.

The pre-syncopal criteria were as follows (Murray et al, 1968);

1. Reductions in SBP of more than 25mmHg or 15mmHg for DBP between successive 1 minute readings;
2. A single SBP reading of less than 80mmHg;
3. A precipitous fall in heart rate of more than 15 beats · min⁻¹;
4. Subject light-headedness, subject nausea or subject request.

Test termination was a rapid (approximately 5 seconds) return of chamber pressure to normal atmospheric pressure. Following test termination all subjects remained in a supine resting state for a further 5-minute control period. All relevant data were recorded from the beginning of the pre-test control period to the end of the post-test control period.

2.6 Data acquisition.

In addition to internal chamber pressure, electrocardiography (ECG), heart rate (HR) and electromyography (EMG) were recorded during orthostatic stress test procedures at a sampling frequency of 1 kHz using an analogue to digital converter (PowerLab, ADInstruments, UK) with appropriate data acquisition software (Chart version 3.4.9 or 4.0.1; ADInstruments, UK).

2.6.1 Electrocardiography (ECG) and heart rate (HR).

A three-lead Bio Amp cable (Tronomed D-1340, ADInstruments, UK) was used in conjunction with three recording electrodes. The electrodes were placed on the 2nd left rib (positive), the left (negative) and right (earth) clavicle. The 'Bio Amp' cable was connected to the PowerLab via a 'Bio Amp' amplifier (ML 132, ADInstruments, UK). The PowerLab was configured to a sampling rate of 1kHz. Using the 'Rate Meter' facility on the PowerLab, instantaneous HR was recorded. This measures the time interval between R-waves from the raw ECG and expresses each R-R interval as HR (beats per minute). Details of the expression of HR responses to orthostatic stress are given in the appropriate methods sections in chapter 3, 4 and 5.

2.6.2 Electromyography (EMG).

A three-lead Bio Amp cable (Tronomed D-1340, ADInstruments, UK) was used in conjunction with three recording electrodes placed on the right leg. Two electrodes were placed on the right vastus medialis muscle (positive and negative) and one on the right patella (earth). The Bio Amp cable was connected to the PowerLab via a 'Bio Amp' amplifier (ML 132, ADInstruments, UK). The facility to sample at different rates between channels was not available and therefore the EMGs were sampled at a rate of 1kHz. In studies where changes in muscle activation were being studied, an integrated EMG signal was calculated using the Chart v4.0.1 software and simultaneously recorded.

2.7 Blood Pressure (BP).

Measures of BP were taken using the oscillometric technique and a pneumatic cuff placed around the upper left arm of subjects. This technique measured the changes in pressure waves from the brachial artery exerted on the pneumatic cuff. The first recorded wave was taken as systolic blood pressure (SBP). Diastolic blood pressure (DBP) was defined as the point at which the pressure waves became constant. All SBP, DBP and mean arterial blood pressures (MAP) were measured and recorded using an automated oscillometric blood pressure monitor (TM-2541R, AND Instruments, UK). It was important to use an automated BP monitor for measurements of BP during orthostatic stress tests, since the vacuum pumps generate a reasonable amount of ambient noise, which has been shown to effect measurement of BP using standard mercury sphygmomanometry and a stethoscope (Lightfoot et al, 1992; Lightfoot et al, 1996). Although similar oscillometric BP monitoring devices have been shown to agree with standard sphygmomanometry (Clark et al, 1991; Imai et al, 1992; Palatini et al, 1998), it was essential to verify this particular device against an established method for measuring BP.

Six subjects (average \pm SD, age 23.7 ± 3.3 years; height 175.1 ± 9.6 cm; mass 67.7 Kg) volunteered to participate in a study that examined the agreement between measures of BP using the oscillometric technique (TM-2541R, AND Instruments, UK) and a standard mercury sphygmomanometer (Accoson, England) and stethoscope (Littmann, USA), after gaining ethical approval. The stethoscope used in this study was of the 'training' type, thus allowing two experimenters to simultaneously listen for Korotkoff (K) sounds. If the two experimenters did not agree on BP measured using

K sounds, then the measurement was repeated. All measures of BP were made with subjects in a seated position. BP using the oscillometric device was measured from the right Brachial artery and BP measures using the mercury sphygmomanometer were taken from the left brachial artery. BP recordings using the two methods were measured simultaneously to avoid small changes in BP affecting the results.

The average difference (\pm SD) between the two methods was 0.6 (\pm 3.3) mmHg for SBP and 3.0 (\pm 3.8) for DBP. Agreement between these two methods for measuring BP was quantified by plotting the differences between the methods, against the average of the two methods at each measurement (Bland and Altman, 1986; Lamb, 1998). Figure 2.5 shows that over 95% of the measures of both SBP and DBP were within + or – 2 SDs of the average difference between the two methods. Therefore, there was an acceptable level of agreement between the two techniques.

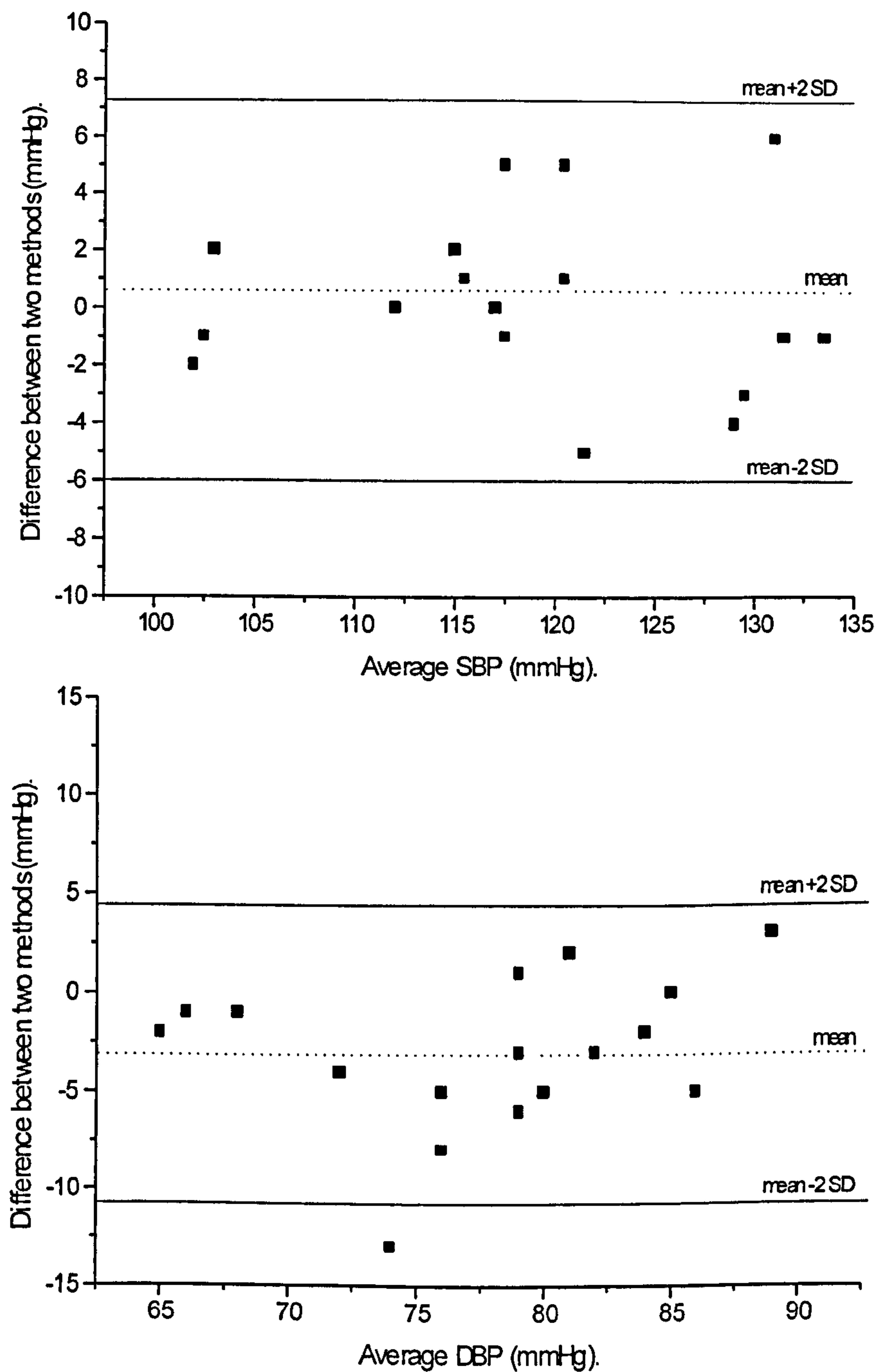


Figure 2.5 Difference between the two methods of BP measurement (oscillometric and mercury sphygmomanometry) plotted against the average of the two methods for each simultaneous measurement made. The top panel shows the level of agreement in measures of SBP and the bottom panel shows the level of agreement in measures of DBP, between the two methods.

2.8 Procedure for collection of blood samples.

The Physiology of Exercise laboratories, De Montfort University, Bedford guidelines for the sampling and handling of blood samples were adhered to for the collection of all blood samples.

2.8.1 Finger prick samples.

Arterialised finger prick samples of blood were taken prior to some orthostatic stress tests (chapters 3 and 5) and placed in duplicate in a micro-haematocrit capillary tube. The capillary tube was then centrifuged at 12,000 rpm for 3 minutes (Hawksley and Sons Ltd, Sussex, UK). Readings of haematocrit (Hct) were taken using a micro-haematocrit reader (Hawksley and Sons Ltd, Sussex, UK). In addition, duplicate blood haemoglobin microcuvette (Hemocue, Sweden) samples were taken to provide a measure of haemoglobin (Hb) using a haemoglobin photometer (Hemocue, Sweden). Measures of Hct and Hb were used to calculate estimated percentage changes in blood and plasma volume between tests, using established methods (Dill and Costill, 1974; chapter 3).

2.8.2 Venepuncture samples.

Samples of venous blood (10mL) were collected from a prepared antecubital fossa into two (2x 5mL) vacutainers containing lithium heparin (BD Vacutainer Systems, UK). In studies where venous blood samples were necessary (see 3.2), additional finger pick samples were avoided by sampling for Hct and Hb directly from the

vacutainers. Venous blood was then centrifuged at 3,000 rpm for 10 min and the plasma was decanted into eppendorf cuvettes (1.5mL) and stored at -80°C until analysis.

2.9 Procedures for urine sample collection.

Urine samples were collected when subjects attended the laboratory for testing (see 3.2). Although this protocol was perhaps not as desirable as first morning excretion, it was at least standardised by collecting urine at the same time of day to account for any possible circadian variation. Samples were mid-stream urine collected into a sterile urine sample container and immediately frozen at -20°C until analysis.

2.10 Determination of sodium (Na^+) concentration in urine and plasma.

Urine Na^+ (U_{Na^+}) and plasma Na^+ (P_{Na^+}) concentrations were determined using a flame photometer (Gallenkamp, UK). Samples of urine (10 μL) and plasma (10 μL) were placed in solution with 5 ml of distilled water. Samples were drawn through a microcapillary tube into a non-luminous flame and were subsequently ionised. The subsequent light emission was detected by a photocell through an appropriate filter. A sample from each solution was analysed and values for Na^+ were calculated from the photometer readings taken from known standards, of 100, 120, 140 and 160 $\text{mmol}\cdot\text{L}^{-1}$, immediately prior to analysis, following transposition of the regression equation $y=a+bx$ to $(y-a)/b=x$ (figures 2.6 and 2.7).

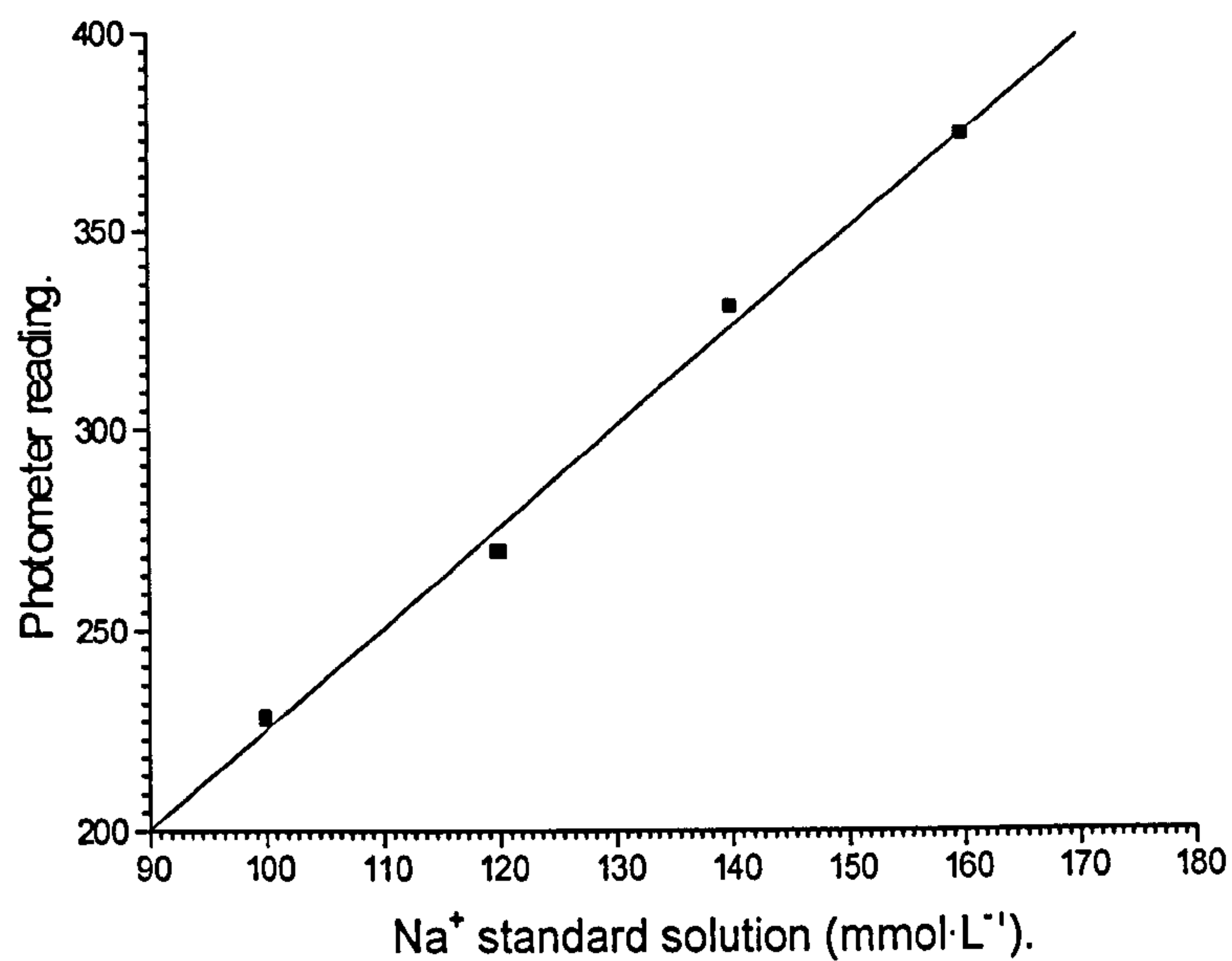


Figure 2.6 Relationship between flame photometer reading and standard solutions of Na⁺ immediately prior to measurement of urine Na⁺ concentration ($r = 0.99$; $P < 0.01$).

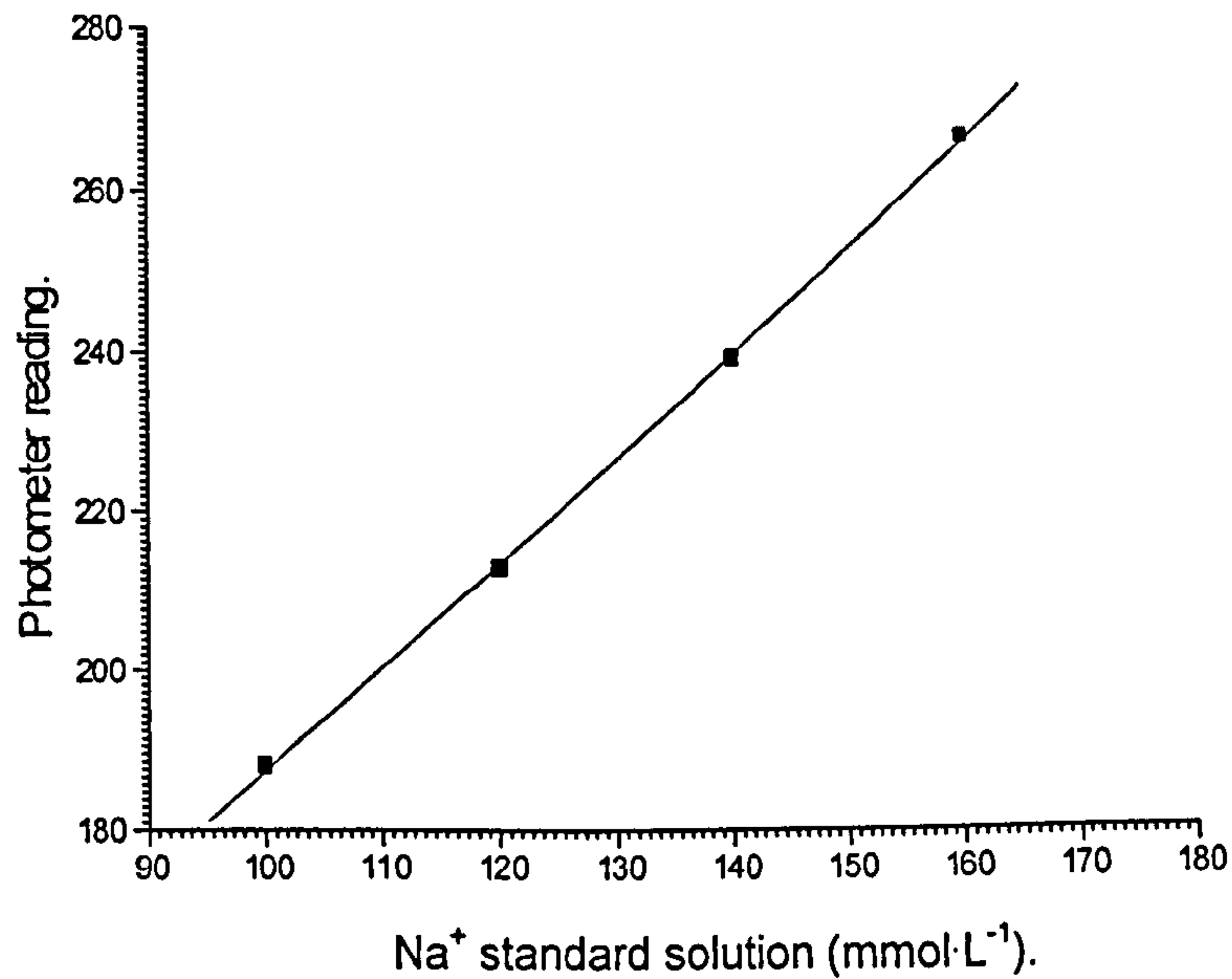


Figure 2.7 Relationship between flame photometer reading and standard solutions of Na⁺ immediately prior to measurement of plasma Na⁺ concentration ($r = 0.99$; $P < 0.01$).

2.11 Determination of creatinine (Cr) concentration in urine and plasma.

Urine creatinine (U_{Cr}) and plasma creatinine (P_{Cr}) concentrations were determined by spectrophotometry (Shimadzu, Japan) using procedures (Sigma Diagnostics, UK. Procedure No. 555) modified from those reported by Heingegard and Tiderstrom (1973), where colour produced by creatinine in the Jaffé reaction was removed by addition of a sulphuric and acetic acid solution.

0.3ml samples of either plasma or urine were prepared in a 1 in 10 dilution with distilled water. 3.0ml of alkaline picrate solution was then added to samples and a known standard solution containing creatinine, before being allowed to stand for 10 minutes at room temperature. Absorbance was measured at 500nm before adding 0.5ml of sulphuric acid and acetic acid mixture to the samples. Samples were then allowed to stand for a further 5 minutes at room temperature before absorbance was again measured at 500nm.

Plasma or urine creatinine concentrations were determined by the following equation and values were subsequently converted into $\text{mmol}\cdot\text{L}^{-1}$;

$$\frac{\text{sample treated with picrate solution} - \text{sample treated with acid reagent}}{\text{standard treated with picrate solution} - \text{standard treated with acid reagent}} \times 3^*$$

* = concentration of creatinine in known standard in $\text{g}\cdot\text{dL}^{-1}$.

2.12 Fractional excretion of sodium (FE_{Na^+}).

The amount of creatinine (Cr) present in the plasma is proportional to muscle mass, which does not change over a relatively short period of time. Furthermore, creatinine is not reabsorbed from glomerular filtrate by the kidney. Therefore, the amount of creatinine in the plasma or urine remains relatively constant. The ratio between plasma and urine creatinine can then be used to indicate changes in urinary volume. Urinary Na^+ concentration however, may change either by increasing its reabsorption by the kidney or and an increase in urinary volume. Therefore, it is essential to express changes in urinary Na^+ concentration relative to changes in urinary Cr concentration. This technique makes the detection of changes in the handling of Na^+ by the kidney possible without collecting 24-hour urine samples. FE_{Na^+} refers to the total quantity of Na^+ in the glomerular filtrate that is present in the urine (Best and Taylor, 1979).

FE_{Na^+} was calculated using the following equation;

$$FE_{Na^+} = (U_{Na^+} / P_{Na^+}) / (U_{Cr} / P_{Cr}) \times 100$$

2.13 Determination of total plasma protein (TPP) and plasma albumin (PA).

Plasma proteins have an important role in the regulation of the distribution of water between extravascular tissue and blood plasma. Of these plasma proteins albumin is the most profuse and it determines oncotic pressure (Tortora and Grabowski, 1995).

TPP and PA concentrations were also determined by established spectrophotometric techniques and procedures (procedure No 541 and 625 respectively; Sigma Diagnostics, UK). TPP was measured using the biuret reaction (Doumas et al., 1981) and PA was measured using a modified Bromcresol purple (BCP) method (Pinnell and Northam, 1978).

To determine TTP 1.0ml of total protein reagent (see procedure No 541 for reagent composition, Sigma Diagnostics, UK) was mixed with 20 μ L of plasma or the known standard provided, in a suitable test tube. These solutions were then allowed to stand at room temperature for 10 minutes. Absorbance was recorded at 600nm and TTP was calculated in $\text{g}\cdot\text{dL}^{-1}$, using the following equation and subsequently converted into $\text{mmol}\cdot\text{L}^{-1}$;

$$\frac{\text{Treated sample x concentration of standard}}{\text{Treated standard}}$$

Plasma albumin concentration was determined by mixing 1.0ml of albumin reagent (Bromcresol purple) with 10 μ l of plasma or the known standard provided. Absorbance in these solutions was then recorded at 540nm and plasma albumin concentration was calculated in $\text{g}\cdot\text{dL}^{-1}$ using the following equation and was subsequently converted into $\text{mmol}\cdot\text{L}^{-1}$;

$$\frac{\text{Treated sample x concentration of standard}}{\text{Treated standard}}$$

2.14 Assessment of leg volume.

Leg volume was determined using an established anthropometric method (Jones and Pearson, 1969). This method has been shown to agree with measures of leg volume using water displacement techniques (Jones and Pearson, 1969).

Recordings of standing and sitting height, using appropriate stadiometers (Holtain Ltd, UK), were used to calculate $\frac{1}{3}$ subischial height. Figure 2.6 details the seven points at which limb girth was measured. The height of each girth was measured using an anthropometer (Holtain Ltd, UK) so that subsequent girth measurements could be accurately repeated. The volume of each truncated cone (figure 2.8) was computed using the equation $v = \frac{1}{3}h (a + \sqrt{ab} + b)$, where a and b are the calculated top and bottom areas of each truncated cone. Leg fat percentage (age corrected where appropriate) was derived from skin-fold measurements at the anterior and posterior thigh, and the medial and lateral calf (values were the mean of triplicate measurements). These values were then used to calculate estimates of fat free leg volume (Jones and Pearson, 1969; chapter 4).

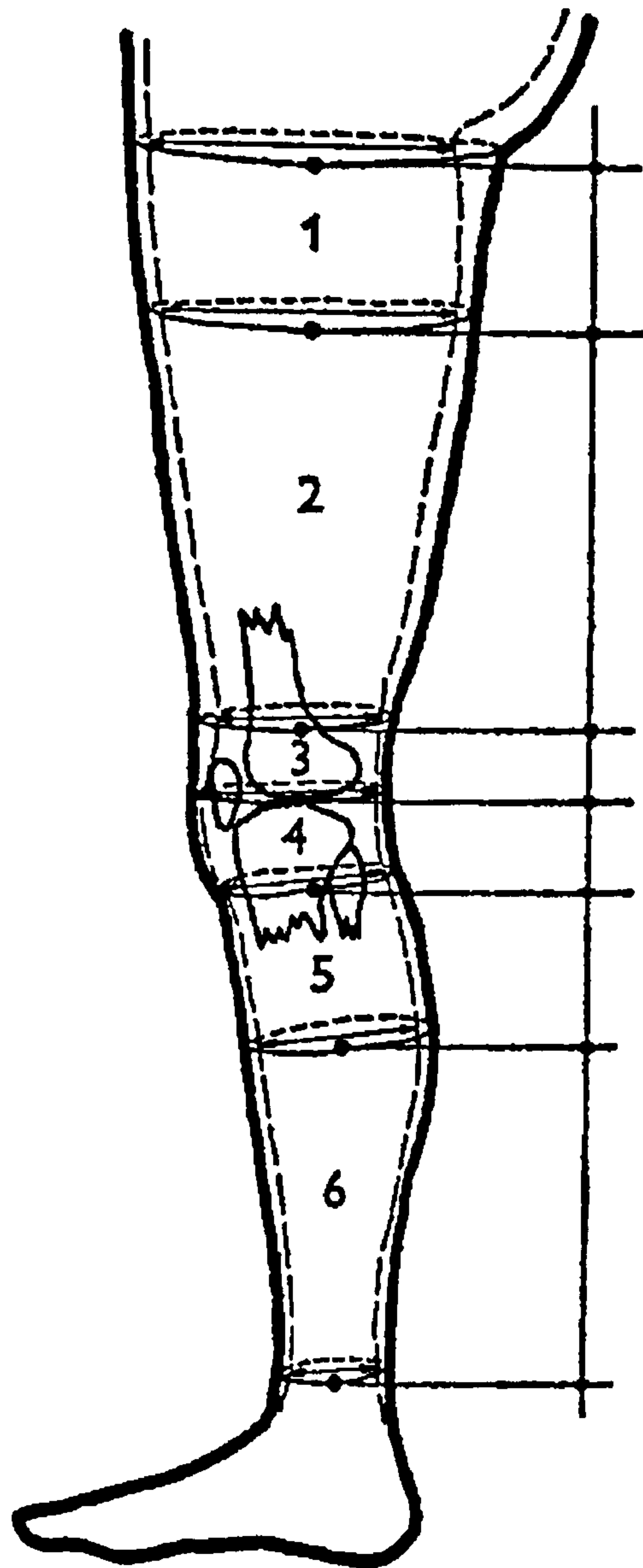


Figure 2.8 Adapted from Jones and Pearson (1969), showing the six truncated cones, the calculated volumes of which were used to assess leg volume.

2.15 Maximum voluntary isometric contraction force (MVC) and isometric exercise.

MVC was determined using an isokinetic dynamometer (Kin-Com, USA), with subjects suitably restrained. For both the arm and leg isometric exercise conditions (see chapter 4), the forearm grip attachment (part #70187) was modified by replacing the rubber grip with a custom made 480 x 30 mm aluminium bar which had been suitably drilled at one end to allow it to fit over the cylindrical section of the forearm grip attachment. This bar was secured to the forearm grip attachment with two hexagonal drive screws. The length of this bar enabled subjects to perform bilateral isometric exercise using either the arms or the legs.

MVC determinations using the legs were performed by contracting the knee extensors at a knee joint angle of 120° (a knee joint angle of 90° was avoided since bilateral maximum force of the legs may have exceeded the 2000N capacity of the dynamometer). MVC determinations using the arms were performed by contracting the elbow flexors at an elbow joint angle of 90°.

There remains a possibility that previously reported reductions in resting BP were associated with the BP response to the isometric exercise (Kiveloff and Huber, 1971; Ray and Carrasco, 2000; Wiley et al, 1992; Peters et al, 2001). Therefore, it was important to assess the differences in the MVC and the BP responses when isometrically exercising at a knee joint angle of 90° and at 120°, particularly since isometric exercise training of the arms was performed at an elbow joint angle of 90°.

Four male subjects (average \pm SD; age 21.5 ± 3.0 years; height 173.6 ± 11.2 cm; mass 64.2 ± 6.3 Kg) performed MVCs using the left knee extensor muscles (for procedure see 2.15) at both joint angles. Subjects then performed an isometric contraction of the knee extensor muscle at 20% of the corresponding MVC at both joint angles for one minute while BP was measured.

MVC was significantly lower when performed with a knee joint angle of 120° (1301 ± 142 vs 905 ± 103 N; Student's paired t test; $P < 0.05$). However, there was not a significant difference in the mean arterial BP (MAP) response, when performing an isometric contraction at 20% of the corresponding MVC, between the two joint angles (figure 2.9).

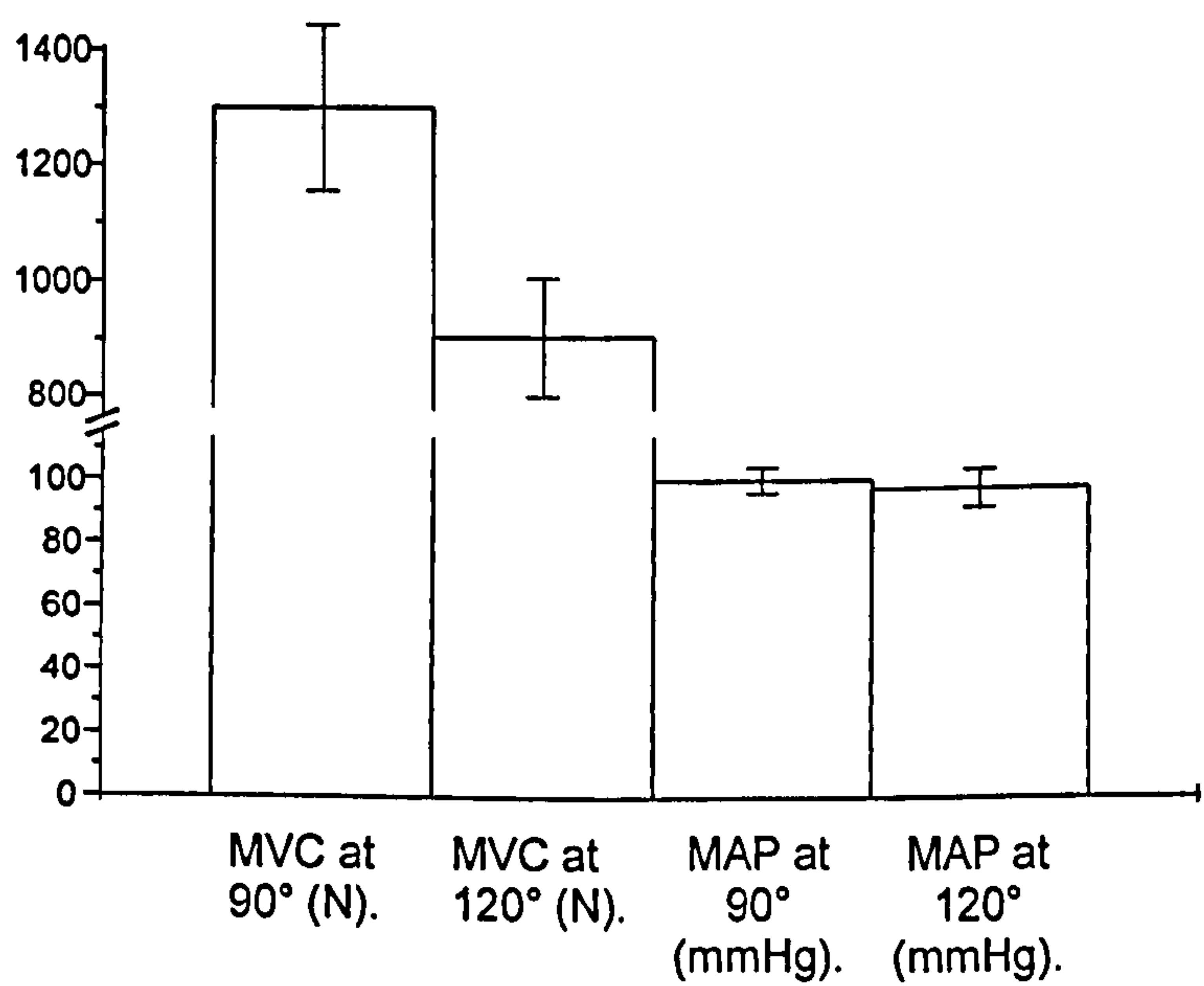


Figure 2.9 Showing MVCs and MAP responses to isometric contractions at 20% MVC. Values are the average \pm SD.

Subjects were required to perform at least two (a maximum of five) MVCs, separated by one minute of rest, that were not different by more than 20%. The maximum force produced from these two MVCs was used to calculate the isometric exercise intensity for that session.

The isometric exercise training protocol used in this study was a modified version of the protocol reported by Wiley et al (1992). In each exercise session, four, two-minute bouts of isometric exercise were performed by subjects at either 30% (arm training) or 20% (leg training) of MVC. Each two-minute bout of exercise was separated by three minutes of seated rest.

2.16 Forearm blood flow.

Forearm blood flow (FBF) was determined indirectly from changes in forearm circumference using forearm occlusion plethysmography (Whitney, 1953). Whitney (1953) concedes that changes in the approximately circular circumference of the forearm when doubled, are equal to changes in the area of the cross-sectional segment around which the measurement of circumference changes are being made. Furthermore, since it is assumed that during changes in limb volume there are no changes along the skeletal axis of the limb, changes in cross-sectional area are equal to changes in limb volume. According to Whitney (1953), if a limb segment with a circumference of 25 cm has a blood flow of $1\text{mL}\cdot\text{dL}\cdot\text{min}^{-1}$, then during venous occlusion the circumference of that segment will increase $0.02\text{ mm}\cdot\text{sec}^{-1}$. Thus FBF

can be calculated from measures of the limb girth and the change in circumference during venous occlusion.

2.16.1 Custom built system for determination of forearm blood flow.

Changes in limb circumference can be determined by recording changes in strain gauge length and thus limb circumference, during venous occlusion. Changes in strain gauge length were recorded, using a Wheatstone bridge amplifier (custom built) and an analogue to digital converter (PowerLab, ADInstruments, UK), as a change in the millivoltage (mV) signal from the strain gauge as its length changes (i.e. a change in the resistance to electrical flow through the mercury in the strain gauge). During venous occlusion the change in the mV signal results in a slope, the mean of which can be measured using the software (Chart version 4.0.1; ADInstruments, UK) accompanying the analogue to digital converter. When analysing slopes from strain gauge signal changes, movement artifact generated on occasion from the abrupt inflation of the occluding cuff (Greenfield, 1963; see 2.16.3) were carefully avoided when calculating strain gauge signal slopes.

2.16.2 Calibration of mercury-in-silastic strain gauge.

Since the depth of the thread on the tension adjustment screw on the strain gauge bridge was known (i.e. a change in strain gauge length of 0.79375 mm per 360° turn of the screw), the relationship between the change in mV signal and the change in strain gauge length was determined by recording the change in mV signal during one

360° turn of the strain gauge tension adjustment screw, thus allowing calculation of change in strain gauge length per mV signal deflection during venous occlusion. This procedure was carried out following every test during which measures of FBF were required.

FBF was calculated using the following set of equations;

$$(1) \quad \frac{\text{change in gauge length during calibration}}{\text{forearm circumference}} \times 100 = a$$

$$\therefore \% \text{ change in forearm circumference} \cdot 1\text{mV}^{-1} = \frac{a}{\text{mV deflection during calibration}}$$

$$(2) \quad \% \text{ change in volume} \cdot 1\text{mV}^{-1} = \% \text{ change in forearm circumference} \cdot 1\text{mV}^{-1} \times 2.$$

$$(3) \quad \therefore \text{FBF (mL} \cdot \text{dL} \cdot \text{min)} = \text{signal slope during occlusion (mV} \cdot \text{s}^{-1}) \times \% \text{ change in volume} \times 60$$

2.16.3 Procedures for measures of forearm blood flow during tests.

During tests a mercury-in-silastic strain gauge was placed on the right forearm, distal to the lateral humeral condyle at the point of maximum forearm circumference. The right arm was supported so that the strain gauge was slightly above the level of the heart (figure 5.1). A small pneumatic cuff placed around the right wrist of the subject was inflated to a suprasystolic pressure to exclude circulation to the hand in the measurement of FBF. A second pneumatic cuff, placed around the upper right arm,

which was inflated abruptly to 50 mmHg, thus occluding venous drainage from the arm. Slopes, during 8 seconds of venous occlusion, from the strain gauge were recorded during five repeated exposures to changes in neck chamber pressure (see 5.1) or every third minute during orthostatic stress tests (see 5.2).

2.17 Determination of changes in calf circumference.

Calf circumference was calculated from changes in the signal output, from a mercury-in-silastic strain gauge placed around the right calf at the point of maximum circumference, during supine rest and during each stage of orthostatic stress in 5.2 (see 2.5). The strain gauge was calibrated at each test as described above (2.16.2) and the subsequent analogue signal from the gauge was recorded as described in 2.16.1. The right calf was slightly elevated to avoid contact between the strain gauge and the foam padding inside the LBNP chamber.

Changes in calf circumference were calculated using the following equations;

$$(1) \quad \frac{\text{mV deflection during calibration}}{\text{change in gauge length during calibration}} = \text{mV signal change per 1mm change in gauge length}$$

$$(2) \quad \therefore \% \text{ change in calf circumference} = \frac{\text{change in mV signal from baseline}}{\text{mV signal change per 1mm change in gauge length}} + \text{pre-test calf circumference}$$

2.18 Application of negative (suction) and positive pressure to the neck.

To alter the distending pressure of the carotid sinus and thus change the level of stimulation to the carotid sinus baroreceptors, varying degrees of suction and pressure were applied to the neck (see 5.2). Two separate neck chambers were used.

Neck suction was applied using a modified version of the chamber described by Eckberg et al (1975). This chamber consisted of a lead chamber shaped to fit between subjects' sternum and clavicles, and the lower part of the mandible. The chamber was secured by a strap placed around the rear of the neck which was fastened to the chamber by the use of velcro. Internal negative chamber pressure was generated abruptly by a rheostatically controlled electric vacuum pump (custom built). Internal chamber pressure was monitored using an analogue sphygmomanometer (Tucos, USA).

Neck pressure was applied using a chamber similar to that described by Sprenkle et al (1986). Using this chamber pressure was applied to the neck by the abrupt inflation of an air tight bladder that was positioned between the rigid chamber and the subject's neck. The chamber was secured to the anterior portion of the subject's neck in the manner described above. Changes in chamber pressure were generated and monitored as described above, except the vacuum pump was modified to introduce air into the chamber as opposed to its removal when negative pressures were required.

2.19 End-tidal CO₂ and minute ventilation analysis.

End-tidal CO₂ concentration and minute ventilation were determined using breath-by-breath mass spectrometry gas analysis (Pulmolab EX 670, Morgan Medical Ltd, UK). Expired gases were drawn through a micro-capillary tube (2.5 m in length) into a vacuum chamber. In this chamber the samples were ionised and molecules were passed through a magnetic field and subsequently separated according to their charge-to-mass ratio and analysed by a specific detector. For a detailed description of the principles of mass spectrometry refer to Chernushevich et al (2001). Immediately prior to each test period the instrument was calibrated using a known certificated (BOC gases, UK) gas mixture (Oxygen 15.00%; Carbon dioxide 5.06%; Argon 5.00%; Nitrogen Bal).

Minute ventilation was determined using the turbine device accompanying the PulmoLab EX-607 (Morgan Medical Ltd, UK; figure 2.10). One full 360° turn of the airscrew in the turbine device is generated when 2.2mL of air passes across it, across a wide range of flow rates. The detector then produces an analogue signal of the number of turbine revolutions with each breath and thus minute ventilation was subsequently calculated using the software accompanying the PulmoLab EX-670 (Exercise Core Interface Application, v 1.2.0.1, Morgan Medical Ltd, UK). The signal from the detector (figure 2.10) was calibrated immediately prior to each test using a 3L syringe (Hans Rudolph, USA).

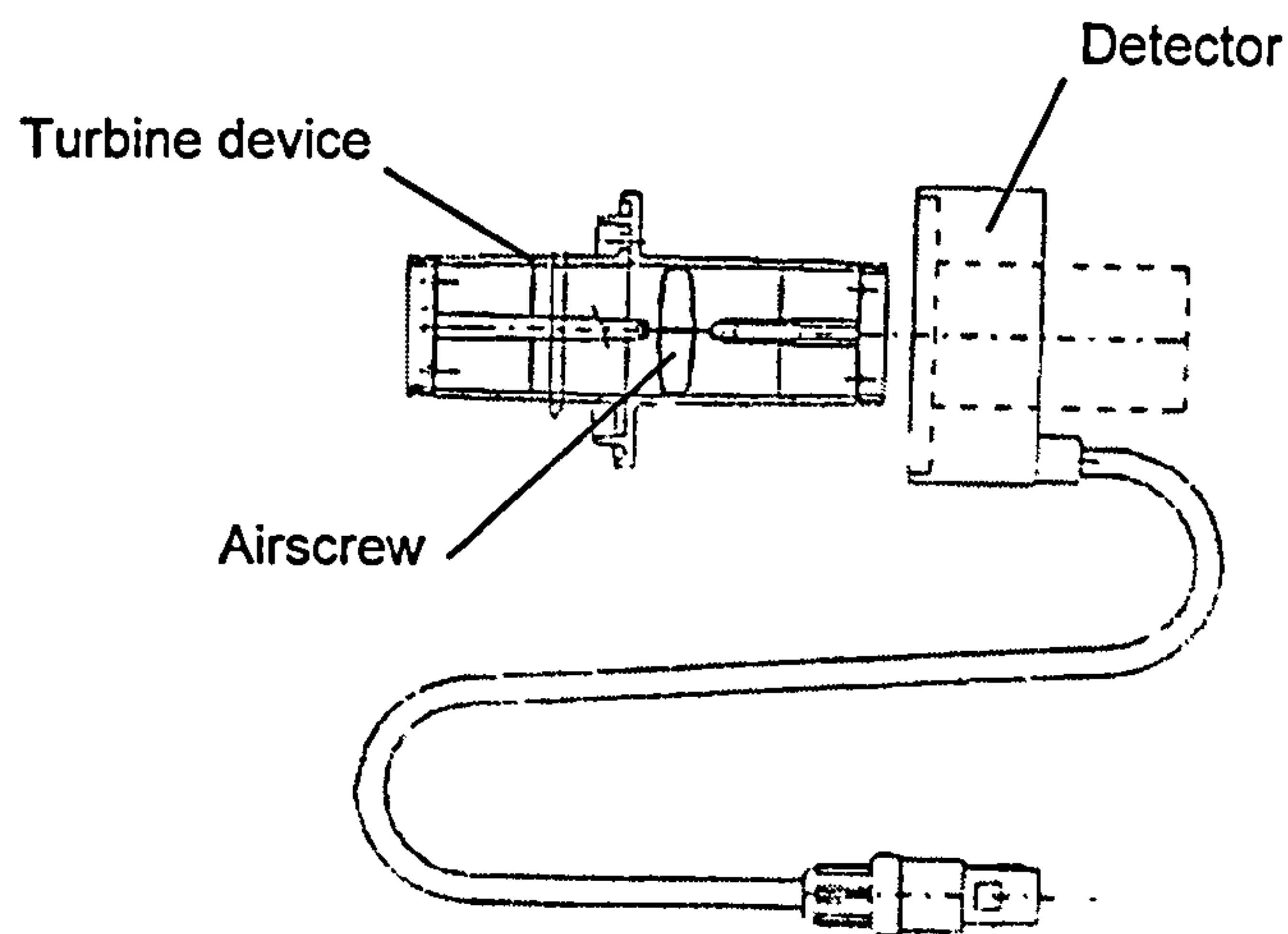


Figure 2.10 Turbine device and detector.

2.20 Estimates of stroke volume and cardiac output.

Estimates of stroke volume (SV) and cardiac output (\dot{Q}) were made using measures of changes in thoracic bioimpedance (Rheocardiomonitor, Rheo-Graphic PTE, Singapore) recorded from surface electrodes. This device has been shown previously to estimate values for SV and \dot{Q} that were in agreement with invasive thermodilution techniques (Barin et al, 2000). The placement of the electrodes and their role in the estimates of SV and \dot{Q} are shown in figure 2.11. 100 kHz 2mA current was applied to the subjects' thorax from electrode I_1 to electrode I_2 . The analogue signal of the impedance of this current was sampled at 200Hz using the U_1 and U_2 electrodes, which also sampled the ECG used in these estimates.

SV was estimated using the following altered Kubicek equation;

$$SV = K \cdot \rho (L/Z_0)^2 \cdot (ELVET \cdot (dZ/dt)_{\max} + Z_{s-q})$$

Where K accounts for physical variations between subjects (e.g. sex, mass, height and chest circumference); ρ is the blood specific resistivity; L is the distance between electrode U_1 and U_2 ; Z_0 is the baseline thoracic impedance; ELVET is the effective left ventricular ejection time; dZ/dt_{\max} refers to the change in Z_0 per unit change in time; Z_{s-q} refers to the change in Z_0 between the Q and S waves in the ECG. A schematic representation of these events can also be seen in figure 2.11. Cardiac output (\dot{Q}) was automatically calculated by multiplying SV and heart rate. A full description of this device and the methods for the calculation of SV and \dot{Q} has been reported previously (Barin et al, 2000).

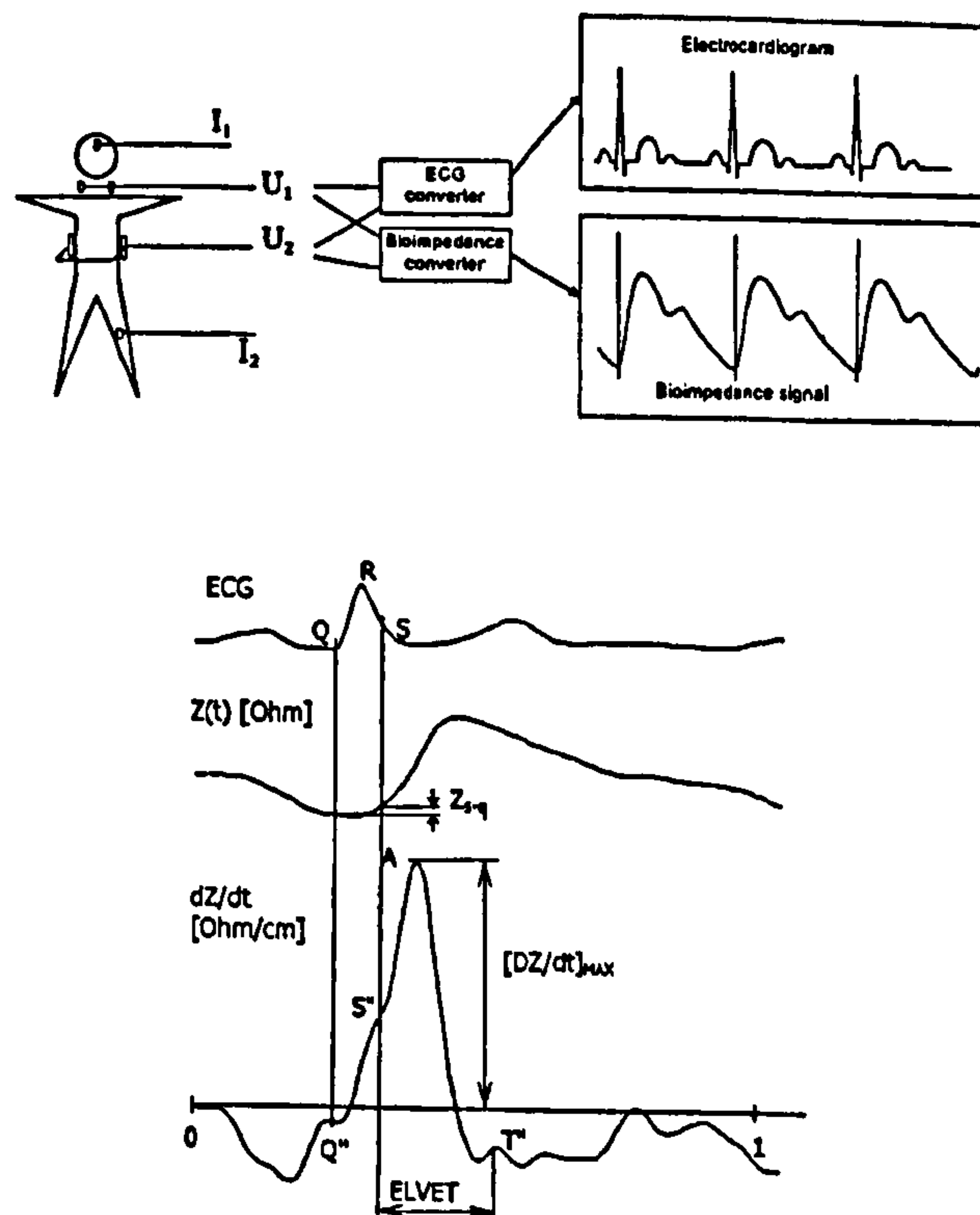


Figure 2.11 Adapted from Barin et al (2000). The top panel shows the placement of the six surface electrodes and their subsequent role in producing the ECG and thoracic bioimpedance signals. The bottom panel represents the ECG and thoracic bioimpedance events that are used to estimate SV and therefore Q. Q'' , start of ventricular systole; S'' , opening of the aortic valve; $Z(t)$, bioimpedance signal; Dz/dt , maximum ventricular contraction; T'' , aortic valve begins to close.

The methods described in the preceding chapter were used in the studies detailed in chapters 3, 4 and 5. Any modifications made to equipment or procedures and details of subjects' and their characteristics are described in the methods sections of the relevant chapters. The preceding description of the methods used in this thesis are coherent with previous studies that have assessed human CV control during orthostatic stress.

Chapter 3.

Orthostatic Stress: Reproducibility and quantification of tolerance.

The first study in this chapter has been published as a full paper and as an abstract;

Howden, R., Tranfield, P.A., Lightfoot, J.T., Brown, S.J., Swaine I.L. (2001). The reproducibility of tolerance to lower-body negative pressure and its quantification. *European Journal of Applied Physiology*, 84, 5, 462-468.

Howden, R., Tranfield, P.A., Lightfoot, J.T., Brown S.J., Swaine, I.L. (2001) The reproducibility of tolerance to lower body negative pressure and its quantification in man *The Journal of Physiology*, 531P, pp. 42P

3.1 Reproducibility of tolerance to orthostatic stress and its quantification in humans.

3.1.1 Introduction.

As discussed in chapter 1, lower body negative pressure (LBNP) can provide an effective orthostatic stress, particularly when assessing factors affecting orthostatic tolerance. However, prior to investigations which aim to increase understanding of human CV control during orthostatic stress, it is essential that the reproducibility of tolerance to orthostatic stress is studied. Furthermore, it is equally important to clarify issues relating to the quantification of orthostatic tolerance.

The performance of custom-made LBNP chambers and protocols used to determine tolerance have seldom been assessed. Also, there is not a single objective indicator of the end-point of orthostatic tolerance. The most popular method for determining orthostatic tolerance uses the occurrence of presyncopal signs and symptoms (Murray et al, 1968). These test termination criteria have been reported many times previously (Convertino et al. 1986; Sather et al. 1986; Sander-Jensen et al. 1988; Lightfoot et al, 1989b; Zhang et al. 1999).

There has been a lack of consistency regarding the most appropriate index by which orthostatic tolerance, when using LBNP, can be expressed and differing reports have included: duration of negative pressure (DNP), magnitude of negative pressure (MNP) and the cumulative stress index (CSI; Luft et al. 1976). CSI, described by Luft

et al. (1976), is the cumulative sum of the products of stage duration and absolute negative pressure. This index has been used in many published reports (Convertino et al. 1986; Levine et al. 1991a; Levine et al. 1991b; Lightfoot et al. 1989a; Lightfoot et al. 1989b; Lightfoot et al. 1991; Muentert et al. 2000; Sather et al. 1986; Zhang et al. 1999). Notably, it produces a curvilinear function when related to LBNP protocol stages (of equal decrements and durations). As a reflection of this lack of consistency, some authors have reported all three of the above indices (Lightfoot et al. 1989b; Lightfoot et al. 1991; Zhang et al. 1999).

The lack of derivation of indices for quantification of orthostatic tolerance has added to the confusion regarding the performance of the LBNP chamber and protocol. Lightfoot and Tsintgaris (1995) recently proposed a novel method for quantifying orthostatic tolerance, referred to as the LBNP tolerance index (LTI). LTI is the cumulative sum of the products of stage duration and the *reduction* in negative pressure at each stage (when all stages of LBNP are of equal duration and negative pressure drop).

There has been only one study in which the reproducibility of orthostatic tolerance, using LBNP, has been assessed (Lightfoot et al. 1991). In this study, which used 11 subjects, the orthostatic stress tests were repeated four times with a period of 72 hours between each test. The statistical analysis suggested that there was not a difference between any of the tests. However, from inspection of the data (their table 1), it appeared that orthostatic tolerance varied by approximately 11 - 15% from test to test. Such a degree of variability is larger than the test-to-test reproducibility that is

usually accepted in other physiological measures (e.g. $\pm 3.6\%$ for $\text{VO}_{2\text{ max}}$ using cycle ergometry; Astrand, 1960). No subsequent studies of the reproducibility of orthostatic tolerance, using LBNP, have included LTI. Therefore, the purpose of this study was to quantify the reproducibility of orthostatic tolerance (LBNP), using a variety of indices.

3.1.2 Methods.

Subjects.

Twenty normotensive (average \pm SD; systolic blood pressure (SBP) 132 ± 11 mmHg; diastolic blood pressure (DBP) 73 ± 9 mmHg) adults (14 males and 6 females; 21 ± 2 years; 172.9 ± 13.1 cm; 68.7 ± 11.6 Kg) volunteered to participate in this study. All subjects were medically screened with a pre-participation questionnaire and informed consent was given by each subject to participate. The investigation was granted ethical approval by De Monfort University School of Sport and Leisure Ethics Committee.

Data acquisition.

Electrocardiography (ECG), heart rate (HR), electromyography (EMG) and chamber pressure were recorded at a sampling frequency of 1 kHz using an analogue to digital converter (PowerLab, ADInstruments, Hastings, UK) with appropriate data acquisition software (Chart version 3.4.9; ADInstruments, Hastings, UK). These methods are described in further detail in chapter 2, sections 2.3 and 2.6.1.

Surface EMG recordings were made from each subjects' right vastus medialis muscle. These data were used to indicate the presence of changing muscle tension, which could facilitate venous return. There were no indications of any changes in muscle tension. A more detailed description of this procedure is given in chapter 2, section 2.6.2.

Blood Pressure (BP).

Measures of BP were taken using the oscillometric technique and a pneumatic cuff that was placed around the upper left arm of subjects. SBP, DBP and mean arterial BP were measured and recorded during the last 20 seconds of every minute during tests using an automated oscillometric blood pressure monitor (TM-2541R, AND Instruments, Oxford, UK). A full description of this technique and the limits of agreement when compared with mercury sphygmomanometry are given in chapter 2, section 2.7.

Blood Volume (BV)

Capillary blood samples were used prior to each orthostatic stress test to obtain measures of haematocrit (Hct), using a centrifuge and haematocrit reader (Hawksley and Sons Ltd, UK) and haemoglobin (Hb) (Hemocue, Sweden). These data were used to estimate changes in blood volume between tests using established methods (Dill and Costill, 1974).

Orthostatic stress testing.

Each subject completed three presyncopal symptom limited orthostatic stress tests, following an initial orientation LBNP exposure to a pressure of -40mmHg . At least 72, but no more than 120 hours separated each LBNP test. All orthostatic stress tests were conducted in accordance to procedures described in chapter 2, section 2.5. Subjects were required to maintain their regular diet and level of physical activity during the course of the study.

Statistical Analysis.

Comparisons between group averages for DNP, MNP, CSI and LTI, control HR, SBP, DBP (HR_{control} , SBP_{control} , and DBP_{control} , respectively; all measured during the final 20 seconds of the fourth minute in the pre-test control period) and baseline to peak (Δ) (see below) HR, SBP, DBP (all measured during the final 20 seconds of the second minute of each LBNP stage) from each experimental test were calculated using a one-way repeated measures analysis of variance (ANOVA; table 3.1.1). The sphericity of the data was assessed using Mauchly's test. Mauchly's test of sphericity tests for any differences in the variance of the data between tests or recorded variables. P values less than 0.05 were considered to show a statistically significant difference between given variable averages. From data that produced statistically significant results the Bonferroni test was used to determine which repeated variables were significantly different from each other (table 3.1.2). Bonferroni's test performs pairwise comparisons following an adjustment to the alpha level based on the number of theoretical comparisons between tests or recorded variables. A one-way repeated measures ANOVA was used to compare the measured physiological responses (i.e. HR, SBP and DBP) for each LBNP test at 20 to 90% (10% increments) of maximum LTI (figure 3.1.2). Data are presented as the average \pm SD.

3.1.3 Results.

All orthostatic stress tests were terminated upon evidence of one or more presyncopal signs or symptoms. Representative data, indicative of the final stages and termination of the test for one subject are shown in Figure 3.1.1. From the 60 tests 81% were terminated due to precipitous decreases in either HR, SBP or DBP, or a combination of all three. The remaining 19% of tests were terminated because the subject reported a sensation of nausea. During no test did syncope or vomiting present.

Reproducibility.

There were no statistical differences between tests 1, 2 and 3 in HR_{control} , SBP_{control} and DBP_{control} just prior to orthostatic stress. The changes in HR, SBP and DBP from control to pre-syncope (Δ) were not statistically significant between tests 1, 2 and 3 (table 3.1.1). Analysis of HR, SBP and DBP at 20 to 90% LTI (in 10% increments) revealed no statistical differences between the three tests at any % of maximum LTI ($P>0.05$; figure 3.1.2). The data at 100% of maximum tolerance was not included in this analysis to avoid skewness in the data, since these responses were frequently depressed (i.e. presyncopal symptoms). The changes in estimated blood volume in tests 2 and 3 relative to test 1 were not significant ($P>0.05$).

When expressed as LTI, there were no statistical differences in orthostatic tolerance between the first and second tests (218 ± 8 vs 217 ± 10 $\Delta\text{mmHg}\cdot\text{min}$; $P>0.05$). However, there was a significant difference between the first and third tests (218 ± 8

vs $233 \pm 9 \Delta\text{mmHg}\cdot\text{min}$; $P<0.05$), and between the second and third tests (217 ± 10 vs $233 \pm 9 \Delta\text{mmHg}\cdot\text{min}$; $P<0.05$) (table 3.1.2).

Quantification of LBNP tolerance.

When calculating orthostatic tolerance using CSI, the statistical differences between tests mirrored that of LTI since there were no differences between tests 1 and 2 (894 ± 63 vs $921 \pm 85 \text{ mmHg}\cdot\text{min}$; $P>0.05$). There was a significant difference between tests 1 and 3 (894 ± 63 vs $1023 \pm 84 \text{ mmHg}\cdot\text{min}$; $P<0.05$), and tests 2 and 3 (921 ± 85 vs $1023 \pm 84 \text{ mmHg}\cdot\text{min}$; $P<0.05$; table 3.1.2). However, expressed as DNP or MNP there were no statistical differences in LBNP_{tol} between the 3 tests ($P>0.05$; table 3.1.1).

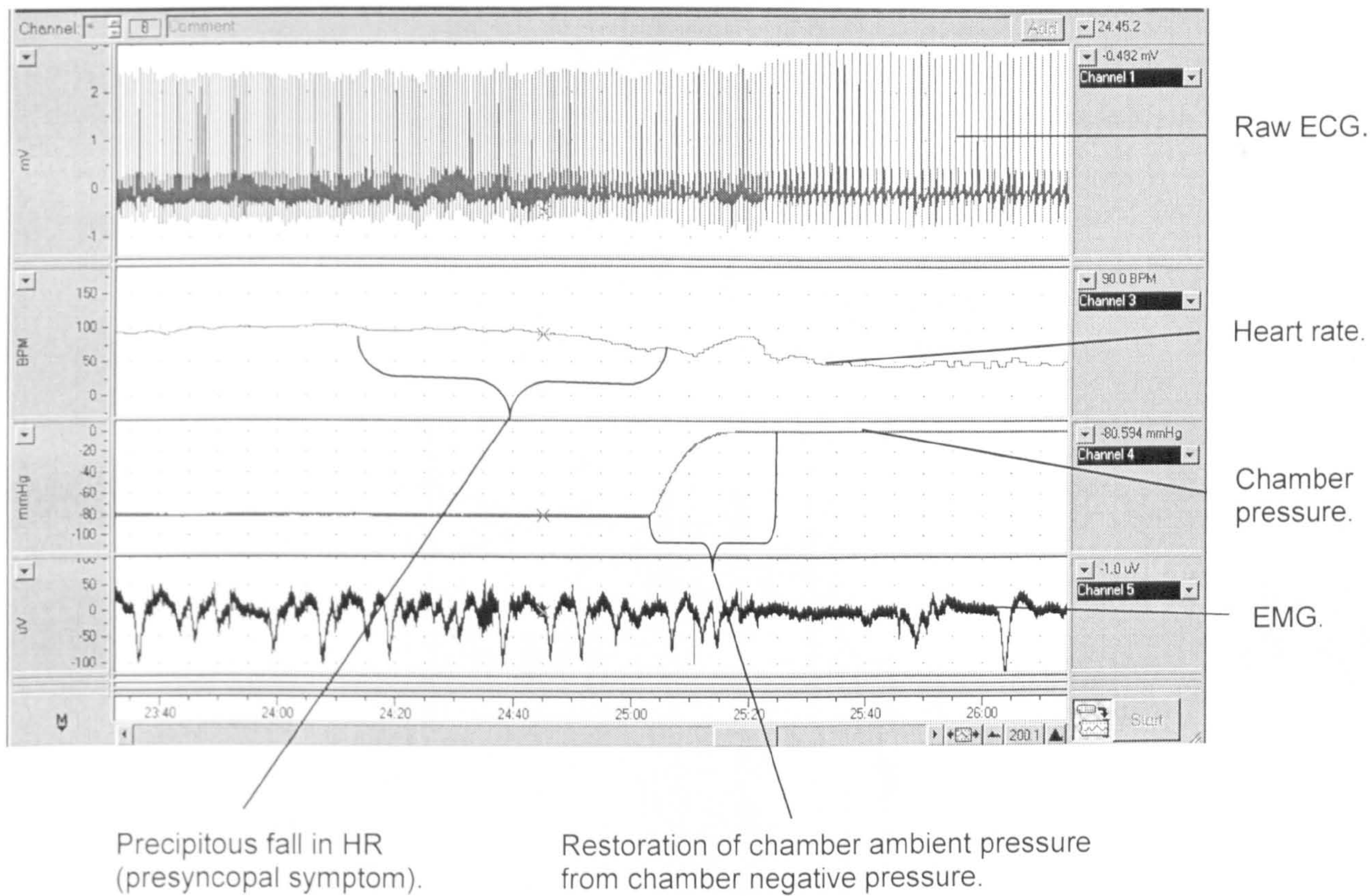


Figure 3.1.1. Raw data form one subject produced by the chart recorder (ADInstruments, Hastings, UK) at the termination of an orthostatic stress test.

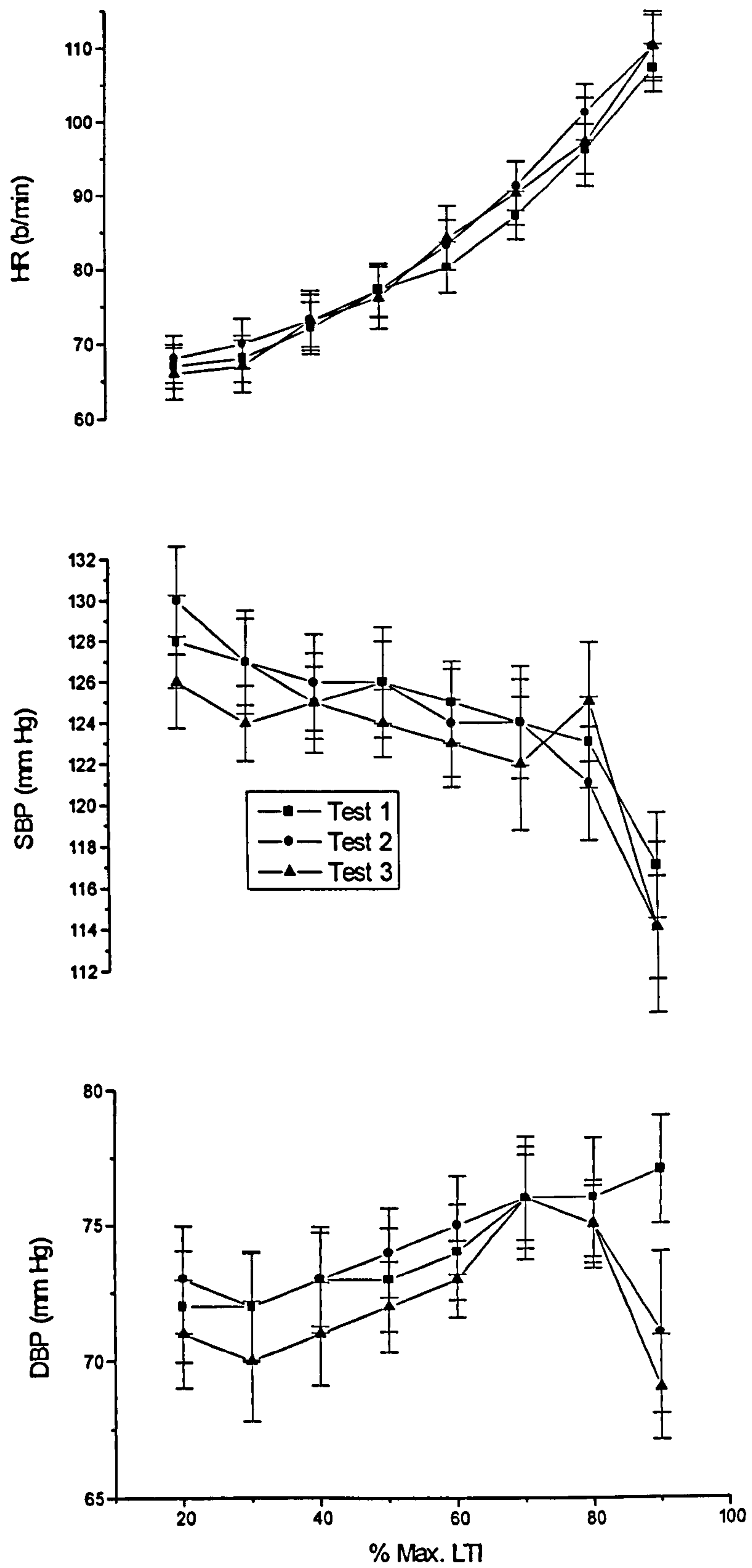


Figure 3.1.2. Heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) responses at 20 to 90% maximum LTI in 10% increments.

Table 3.1.1. Measured physiological response and LBNP tolerance data from the three repeated orthostatic stress tests. Control = pre-LBNP physiological values, Δ = control to peak LBNP responses of measured variables and LBNP tolerance scores. ΔmmHg = change in negative pressure from one stage to another. ΔBV = % changes in estimated BV relative to test 1. Values are average ± SD; n = 20. * = significant difference between tests averages, therefore the Bonferroni test was used on this data (see below). P value = degree of probability following the comparison of the above data for each of the three repeated tests

Variable	LBNP test.			
	1	2	3	P value
Control HR (b·min)	66 ± 12	67 ± 13	66 ±14	0.66
Control SBP (mmHg)	132 ± 11	133 ± 10	130 ± 10	0.19
Control DBP (mmHg)	73 ± 9	73 ± 7	72 ± 8	0.66
ΔHR (b·min)	58 ± 11	61 ± 16	60 ± 20	0.70
ΔSBP (mmHg)	- 40 ± 23	- 33 ± 22	- 39 ± 23	0.52
ΔDBP (mmHg)	- 21 ± 19	- 14 ± 20	- 20 ± 17	0.31
LTI (ΔmmHg · min)	218 ± 37	217 ± 46	233 ± 42	0.01*
CSI (mmHg · min)	894 ± 284	921 ± 379	1023 ± 374	0.02*
DNP (min)	18.8 ± 3.8	18.9 ± 4.7	20.2 ± 4.8	0.06
MNP (mmHg)	78 ± 13	76 ± 16	81 ± 14	0.08
Δ BV (%)	0	1	0.1	0.76

Table 3.1.2. Pairwise Comparisons showing which orthostatic stress tests were significantly different from each other following the employment of the Bonferroni test. * = P values less than 0.05 which were considered to show a statistically significant difference between tests.

Tolerance index.		Test.	
LTI (Δ mmHg · min)	1	2	
	1	3*	
	2	3*	
CSI (mmHg · min)	1	2	
	1	3*	
	2	3*	

Table 3.1.3. LBNP tolerance index scores for the twenty subjects in tests 1-3. Scores are LTI ($\Delta mmHg \times min$) for each subject in tests 1, 2 and 3.

Subject	Test 1.	Test 2.	Test 3.
1	212.77	194.34	184.46
2	226.09	203.04	224.17
3	213.01	206.73	219.26
4	202.46	219.99	184.58
5	163.96	164.16	193.27
6	240.30	237.74	259.93
7	205.29	194.74	198.24
8	285.18	320.35	332.93
9	245.61	219.62	228.28
10	194.19	204.27	222.02
11	198.11	231.15	251.77
12	132.17	121.19	184.07
13	213.35	248.28	237.61
14	249.60	248.48	253.61
15	200.42	153.90	197.30
16	285.01	265.79	296.67
17	192.90	208.23	192.86
18	255.14	287.37	285.61
19	224.03	176.80	231.77
20	222.70	235.21	272.99

3.1.4 Discussion.

This study examined the reproducibility of orthostatic tolerance using a custom built LBNP chamber. There were statistically significant differences in orthostatic tolerance when using LTI and CSI as LBNP tolerance indices between tests 1 and 3, and 2 and 3. Tests 1 and 2 were almost identical. Further, there were no differences in control or responses of HR, SBP or DBP to three successive presyncopal symptom limited exposures to orthostatic stress.

Quantification of orthostatic tolerance.

DNP and MNP showed no significant difference between the three orthostatic stress tests conducted in this investigation. DNP is perhaps a useful index when exposing subjects to a constant level of orthostatic stress and recording the time to pre-syncope symptoms. However, many protocols expose subjects to increasing levels of orthostatic stress. This incremental stress was not reflected in DNP and must be taken into account since the level of intravascular fluid movement is positively related to the magnitude of negative pressure (Cleroux et al, 1989). Conversely, MNP does take into account the maximum level of orthostatic stress tolerated. However, individuals may tolerate similar negative pressure, for different durations. Under these circumstances, MNP may not be used to distinguish between subjects. Thus, an index calculated from the level of negative pressure and duration of exposure to that pressure would be more sensitive to differences in orthostatic tolerance.

The CSI combines DNP and the increasing levels of negative pressure. Luft et al. (1976) noted that CSI was curvilinear even if stage duration and decrements in negative pressure were uniform throughout the LBNP protocol and suggested that this would correlate with physiological changes during orthostatic stress. However, recent work by Lightfoot and Tsintgaris (1995) suggests that the CSI does not adequately reflect important physiological responses to orthostatic stress, such as volume of fluid sequestered or central venous pressure, which are fairly linear up to –60mmHg. However, this index did show a higher degree of sensitivity than MNP and DNP by a significant difference between the first two tests and test 3.

There are no published reports that have used LTI as an index for tolerance to LBNP. Authors continue to report CSI (Engelke et al, 1996; Zhang et al, 1999; Muentner et al, 2000) despite a number of problems associated with its use being proposed by Lightfoot and Tsintgaris (1995). Most LBNP protocols incorporate equal stage durations and reductions in negative pressure. Therefore, the ‘stress’ applied appears to be linear and hence it appears appropriate to use an index that produces a linear function under such conditions. The data of the present study supports the usefulness of LTI, since this index was sensitive to differences in tolerance between the repeated tests whilst retaining its linear relationship to increases in orthostatic stress. As suggested by Lightfoot and Tsintgaris (1995), the curvilinear function of CSI appears to magnify differences in tolerance between individuals. An individual who could tolerate LBNP to –80mmHg, would appear to be 260% more tolerant than an individual who could tolerate only –40mmHg (only half the ‘stress’). In table 3.1.1, using LTI the group increase in maximum tolerance between tests 1 and 3 is 6.4%.

However, the supposed increase between the same tests using CSI is almost twice this value (12.6%).

Reproducibility

The difference found in LTI partly contrasts with the work of Lightfoot et al. (1991), who found that the differences in four repeated orthostatic stress exposures were not statistically significant in 11 subjects. Suggested explanations for increases in orthostatic tolerance, in the absence of any exogenous treatment to subjects, include altered plasma volume regulation (Lightfoot et al, 1989) and the formation of oedema, thus affecting lower limb compliance (Hilton et al, 1988).

In the present study 11 (55%) of the subjects were more tolerant in the third test relative to the first two tests (table 3.1.3). Indeed, 2 of these subjects showed a substantial (16 and 30% for subjects 20 and 12 respectively; table 3.1.3) increase in tolerance during the third test. If these two subjects were removed from the analysis, the remaining eighteen subjects show no significant difference between all three tests. However, there was no justifiable reason to exclude these subjects from the study and therefore it appears that orthostatic tolerance can vary quite considerably when three orthostatic stress tests are performed within a narrow time period. The increase in tolerance may be attributed to an unknown characteristic in the cardiovascular systems of the present sample that was not evident in the subjects of Lightfoot et al. (1991). It is also possible that the larger group presented here, better represents the level of reproducibility in orthostatic stress testing. In addition, since there is no single objective end point indicating orthostatic tolerance. Many authors

have reported a number of pre-syncopal signs and symptoms that have been used either individually or in combination as indicators of pre-syncope (Convertino et al, 1986; Sather et al, 1986; Sander-Jensen et al, 1988; Zhang et al, 1999). Therefore, it is possible that the ambiguity associated with orthostatic stress test termination, contributed to the variability found here.

In the present study, results suggest that in a test-retest experimental design there is an acceptable level of reproducibility when exposing subjects to two repeated orthostatic stress tests within a short period (i.e. 72-120 hours). To avoid adaptive increases in tolerance (Lightfoot et al, 1989) it has been suggested that a period of 72 hours between orthostatic stress exposures is required (Lightfoot et al, 1991). However, since 55% of the sample used in the present study showed a systematic increase in tolerance to LBNP, it is possible that a period of 72-120 hours between tests is not sufficient to prevent systematic increases in orthostatic tolerance if more than two sequential orthostatic stress exposures are administered.

In attempting to explain the variation in orthostatic tolerance found using the present chamber and protocol, it is tempting to compare the physiological responses of subjects at given absolute negative pressures. One method to achieve this is to analyse the measured physiological parameters during each orthostatic stress test stage up to and including the negative pressure that all subjects tolerated (Lightfoot et al, 1991), in this case – 40 mm Hg. However, at – 40 mm Hg, some individuals would be at their maximum tolerance, whereas others might be comfortably within their capacity. Hence, the degree of cardiovascular stress (in relation to peak

tolerance) would be quite different. Furthermore, if the analysis was continued beyond this commonly tolerated negative pressure (– 40 mm Hg), the number of subjects within the sample would decrease progressively and hence reduce the statistical power.

Perhaps the most appropriate method currently available for comparing physiological responses between repeated tests is to analyse the data at given percentages of LTI (Lightfoot and Tsintgaris, 1995). However, this method of analysis showed no significant differences between tests in the responses of HR, SBP, or DBP at any percentage of LTI (figure 3.1.2) despite differences in LTI. It appears therefore, that the LTI of a subject, at any given point in time probably determines the HR and BP responses to orthostatic stress, rather than *vice versa*. Therefore, this method appears to be useful only when making between-subject, but not within-subject, comparisons.

In order to assess the postulated factors by which increases in orthostatic tolerance occur following repeated exposure to LBNP (Hilton et al, 1988; Lightfoot et al, 1989) and to offer further explain for the increase in orthostatic tolerance found in this study, further investigation is required. Previous reports (Hilton et al, 1988; Lightfoot et al, 1989) have demonstrated increases in orthostatic tolerance when subjects were exposed to orthostatic stress daily. Therefore, to investigate possible alterations in plasma volume regulation by the kidney and the formation of oedema it may be necessary to expose subjects to a series of orthostatic stress tests each separated by 24 hours.

3.1.5 Conclusions.

This study has provided evidence to suggest that twice-repeated orthostatic stress exposures resulted in reproducible measures of LTI for orthostatic tolerance. When more than two orthostatic stress tests were conducted with only a short period (72-120 hours) between tests, an increase in orthostatic tolerance was observed. Therefore, before a study design that involves three or more orthostatic stress tests in short succession can be considered, the time period allowed between tests must be investigated to avoid this increase in orthostatic tolerance if an increased risk of performing a type I error is to be avoided. Possible adaptations that may result from repeated exposure to orthostatic stress have been poorly researched. Hence, these data demonstrate a requirement to investigate possible mechanisms that are responsible for increases in orthostatic tolerance (refer to 5.1). Finally, the present data support the finding of Lightfoot and Tsintgaris (1995) that the LTI is a sensitive and reliable index of tolerance to orthostatic stress, using LBNP.

3.2 Indices of plasma volume regulation during daily exposure to orthostatic stress.

3.2.1 Introduction.

In the preceding section (3.1) three repeated exposures to orthostatic stress, using LBNP resulted in an increase in orthostatic tolerance. Therefore, the aim of the proceeding study was to further examine the effect of repeated orthostatic stress on orthostatic tolerance.

During orthostatic stress, increased transcapillary pressure in the dependant limbs has been known to induce a loss of plasma water into interstitial space (Fawcett and Wynn, 1960; Hilderbrandt et al, 1993; Brown and Hainsworth, 1999). Haemoconcentration and increased plasma protein concentration (Noddeland et al, 1981; Lundvall and Bjerkhoel, 1995) have been used as indicators of acute changes in plasma volume. In response to reduced plasma volume during orthostatic stress, retention of sodium and water by the kidney has been reported (Gilbert et al, 1966; Rowell, 1993). Lundvall and Bjerkhoel (1995) reported an incomplete recovery of plasma volume during supine rest after orthostatic stress. Also, Hilderbrandt et al (1993) reported a slower rate of fluid absorption into the vasculature following orthostatic stress when compared to capillary filtration during orthostatic stress. Therefore, it is possible that an accumulation of fluid in the interstitial spaces (oedema) may occur, after repeated exposures to orthostatic stress. Also, sodium

and water retention by the kidney may also be cumulative when orthostatic stress is presented repeatedly.

Studies that have assessed the effect of daily exposure to orthostatic stress have reported substantial improvements in orthostatic tolerance (Hilton et al, 1988; Lightfoot et al, 1989). It has been suggested that mechanisms responsible for the increased tolerance when orthostatic stress was repeated include oedema formation in the lower limbs (Hilton et al, 1988) and altered plasma volume regulation (Lightfoot et al, 1989). The increased orthostatic tolerance may have been related to the distribution of plasma volume and its regulation by changes in sodium excretion by the kidney.

Information regarding changes in the excretion of sodium can be obtained by expressing sodium concentration in urine as a fraction of its concentration in plasma. Fractional excretion of sodium refers to the total quantity of sodium in glomerular filtrate that is present in urine (Best and Taylor, 1979). Therefore, it may be possible to detect changes in sodium excretion without collection of 24-hour urine samples.

The purpose of this study was to assess possible changes in the regulation of plasma volume during repeated exposure to orthostatic stress. Mechanisms for possible changes in orthostatic tolerance during the repeated stress exposures were examined by investigating changes in fractional excretion of sodium, and lower limb oedema indicated by changes in lower limb volume.

3.2.2 Methods.

Subjects.

Nine volunteers (6 males and 3 females; average \pm SD, systolic blood pressure (SBP) 113.9 ± 12.9 mmHg; diastolic blood pressure (DBP) 70.9 ± 10.1 mmHg; age 23.4 ± 5.3 years; height 180.3 ± 7.2 cm; mass 75.7 ± 11.1 Kg) participated in this study, having been granted ethical approval, after giving written informed consent. All subjects were medically screened using a pre-participation questionnaire before testing. During the study, all subjects were required to abstain from consumption of alcohol, exercise, and any form of sexual practice.

Estimates of leg volume (LV).

Upon arrival at the laboratory subjects' left lower limb volume was assessed using the methods of Jones and Pearson (1969). Briefly, limb circumference was determined at seven sites, partitioning the leg into six truncated cones. The volume of each truncated cone was then determined and volumes were then summed to provide a value for total limb volume. A detailed description of this methods is provided in chapter 2, section 2.10.

Collection and storage of urine and blood samples, and measures of haematocrit and haemoglobin.

Following estimates of limb volume approximately 20 mL of mid-stream urine was collected from subjects into a 25 mL sample container prior to orthostatic stress and immediately frozen at -20°C . In one subject a further urine sample was collected

within 10 minutes after each orthostatic stress test to assess possible changes in FE_{Na^+} within the 24 hour period between tests. After 20 minutes of supine rest, samples of venous blood (10mL) were collected from a prepared antecubital vein into two (2x5mL) vacutainers containing lithium heparin (BD Vacutainer Systems, Plymouth, UK). From each vacutainer, haematocrit (micro-haematocrit method, Hawksley and Sons Ltd, UK) and haemoglobin (Hemocue, Sweden) were determined and these data were used to estimate percentage changes in plasma volume using established methods (Dill and Costill, 1974). The remainder of the venous blood was then centrifuged at 3,00 rpm for 10 min and the plasma was decanted into eppendorf cuvettes (1.5ml) and stored at $-80\text{ }^{\circ}\text{C}$ until analysis. Further details of these procedures are given in chapter 2, sections 2.8 and 2.9.

Urine and Plasma sodium (Na^+) and creatinine (Cr), total plasma protein (TPP) and plasma albumin.

Urine Na^+ (U_{Na}) and Plasma Na^+ (P_{Na}) concentrations were determined using a flame photometer (Gallenkamp, Loughborough, UK). Briefly, samples of urine (10 μL) and plasma (10 μL) were placed in solution with 5 ml of distilled water. Duplicate samples from each solution were analysed and values for Na^+ were calculated from photometer readings taken from known standards.

Urine creatinine (U_{Cr}) and plasma creatinine (P_{Cr}) were determined spectrophotometrically (Shimadzu, Kyoto, Japan) using a diagnostic kit (Sigma Diagnostics, UK. Procedure No. 555). Fractional Excretion of Na^+ (FE_{Na^+}) was calculated following analysis of P_{Na^+} , P_{Cr} , U_{Na^+} and P_{Cr} .

Total plasma protein (TPP) and plasma albumin (PA) concentrations were also determined by established spectrophotometric techniques. Total plasma protein was measured using the biuret reaction (Dumas et al., 1981) and plasma albumin was measured using a modified Bromcresol purple (BCP) method (Pinnell and Northam, 1978; Sigma Diagnostics, Poole, UK. Procedures 541 and 625 respectively). These biochemical assays are described in detail in chapter 2, sections 2.11, 2.13 and 2.14.

Orthostatic stress tests.

The LBNP chamber and protocol used in this study have been described in detail elsewhere (chapter 2, sections 2.1 to 2.5). Briefly, subjects remained in the supine position throughout the orthostatic stress test with their lower extremity, to the level of the iliac crests, sealed in the LBNP chamber. Following a 5 minute control period, internal chamber pressure was reduced by 20 mmHg for 3 minutes. After this 3 minute period, internal chamber pressure was further reduced by 10 mmHg every 3 minutes until the onset of presyncopal signs and/or symptoms. Before participating in this study, all subjects completed LBNP exposure to -40 mmHg in order to familiarise themselves with the orthostatic stress test procedures.

Blood pressure (BP), electrocardiography (ECG) and electromyography (EMG).

During the orthostatic stress tests measures of BP were taken using a pneumatic cuff and an automated oscillometric blood pressure recording device (TM-2541R, AND Instruments, Oxford, UK; see 2.7). BP was measured and recorded during the last 20 seconds of each minute during all orthostatic stress tests. Control values for SBP and DBP were averaged from three consecutive measurements recorded

immediately prior to each orthostatic stress test. Blood pressure responses to orthostatic stress were expressed as the difference between control values and the largest reduction in both SBP and DBP (Δ) before pre-syncopal signs and symptoms were evident.

Surface ECG and EMG recordings were made during experiments from a standard three-lead configuration and the right vastus medialis muscle respectively (see 2.6). Heart rate (HR) was calculated by expressing each recorded R-R interval as beats per minute, automatically by the data acquisition software (Chart v 4.0.4; AD Instruments, Hastings, UK; see 2.6.1). Control values for HR were an average of the final minute of the 5 minute pre-LBNP control period. HR responses to orthostatic stress were expressed as the difference between the control values and the maximum recorded HR during orthostatic stress.

Statistical analysis.

The significance of changes in LTI, LV, estimated percentage changes in PV, FE_{Na+} , TTP, and PA were assessed using a one-way repeated measures ANOVA. The sphericity of the data was determined using Mauchly's test. P values of less than 0.05 were considered to show significance.

3.2.3 Results.

Control and response to orthostatic stress values for blood pressure (SBP and DBP) and heart rate (HR).

There were no significant changes in the control values, recorded immediately prior to LBNP, or in the response to orthostatic stress (Δ) of either SBP or DBP ($P > 0.05$; table 3.2.1). HR responses (Δ) and peak HR during orthostatic stress showed no significant changes across the 5 repeated tests. However, there was a significant increase in the values for control HR immediately prior to the fifth orthostatic stress test when compared to immediately prior to the first test ($P < 0.05$; table 3.2.1).

Orthostatic tolerance and fractional excretion of sodium (FE_{Na+}).

Orthostatic tolerance did not change significantly between the 5 repeated tests (average \pm SD; 188.6 ± 43.8 vs 198.5 ± 34.3 vs 207.4 ± 46.5 vs 194.3 ± 22.3 vs 189.7 ± 31.9 LTI; $P > 0.05$). Orthostatic tolerance showed an increasing trend over the first 3 tests and then declined over the fourth and fifth tests (figure 3.2.1). There was no significant change in FE_{Na+} between the 5 orthostatic stress tests (0.71 ± 0.29 vs 0.81 ± 0.40 vs 0.86 ± 0.40 vs 0.65 ± 0.19 vs 0.53 ± 0.23). However, the pattern of small changes in FE_{Na+} (figure 3.2.1) were similar to those of orthostatic tolerance, producing a significant correlational relationship ($r = -0.29$, $P < 0.05$; figure 3.2.2).

Total plasma protein (TPP), plasma albumin (PA), estimated % changes in plasma volume (PV) and leg volume (LV).

Five repeated orthostatic stress tests did not affect total plasma protein concentration (83.59 ± 6.32 vs 82.1 ± 6.4 vs 84.1 ± 5.3 vs 80.0 ± 5.2 vs 85.2 ± 5.9 vs 80.4 ± 10.7 mmol·L⁻¹; $P > 0.05$; figure 3.2.3] or plasma albumin concentration (38.8 ± 4.1 vs 38.7 ± 3.1 vs 39.0 ± 3.2 vs 39.6 ± 2.8 vs 38.9 ± 7.2 mmol·L⁻¹; $P > 0.05$; figure 3.2.3).

Percentage changes in plasma volume, estimated from measures of haematocrit and haemoglobin were also unaffected by the 5 repeated orthostatic stress tests when compared to the first test (table 3.2.1). The lack of changes in plasma proteins or volume was accompanied by a highly consistent daily anthropometric measurement of leg volume with no significant changes ($P > 0.05$; table 3.2.1).

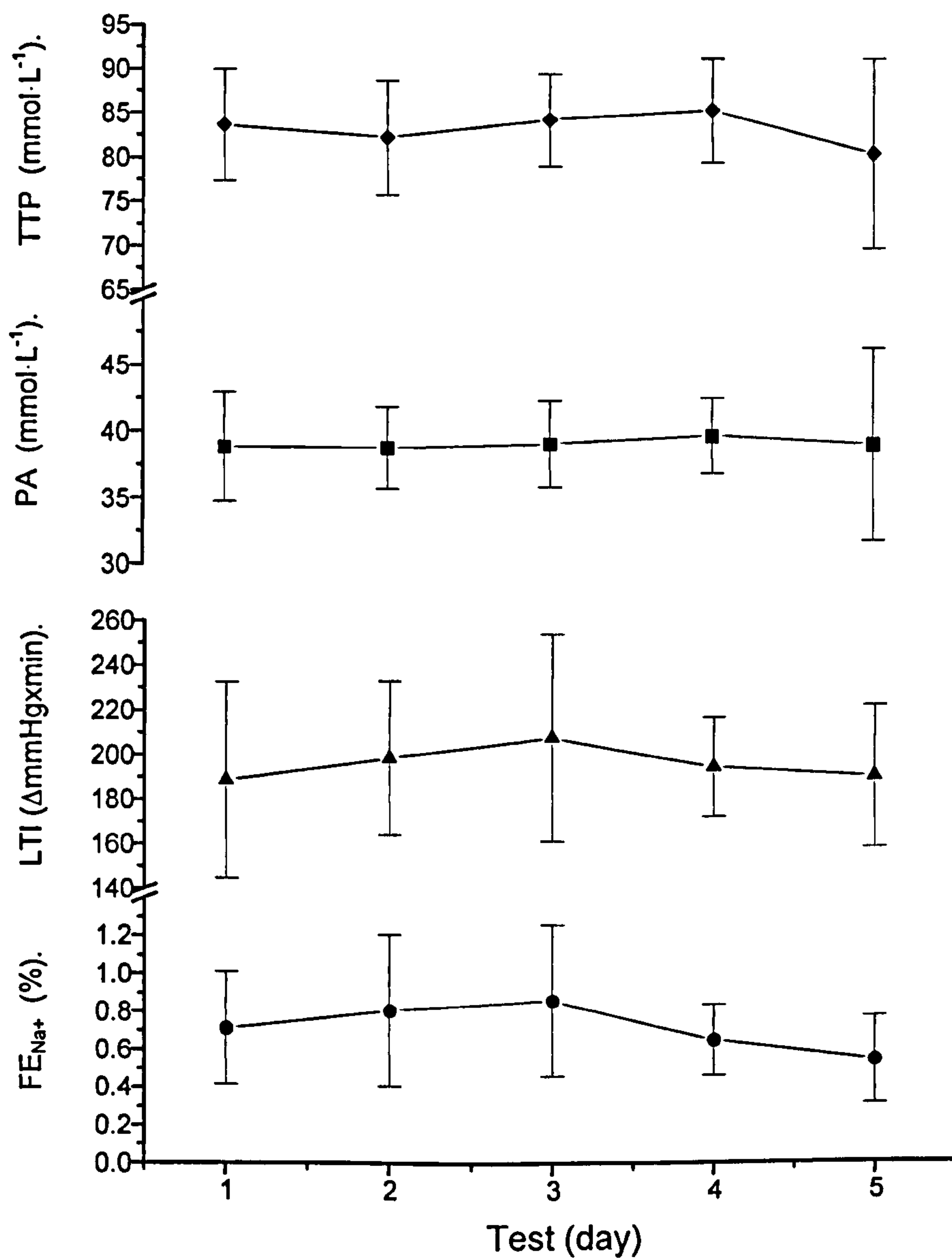


Figure 3.2.1. Total plasma protein concentration (TTP; ◆), plasma albumin concentration (PA; ■), orthostatic tolerance (LTI; ▲) and fractional excretion of sodium (FE_{Na+}; ●) during the 5 repeated exposures to lower body negative pressure. All values are average \pm S.D.

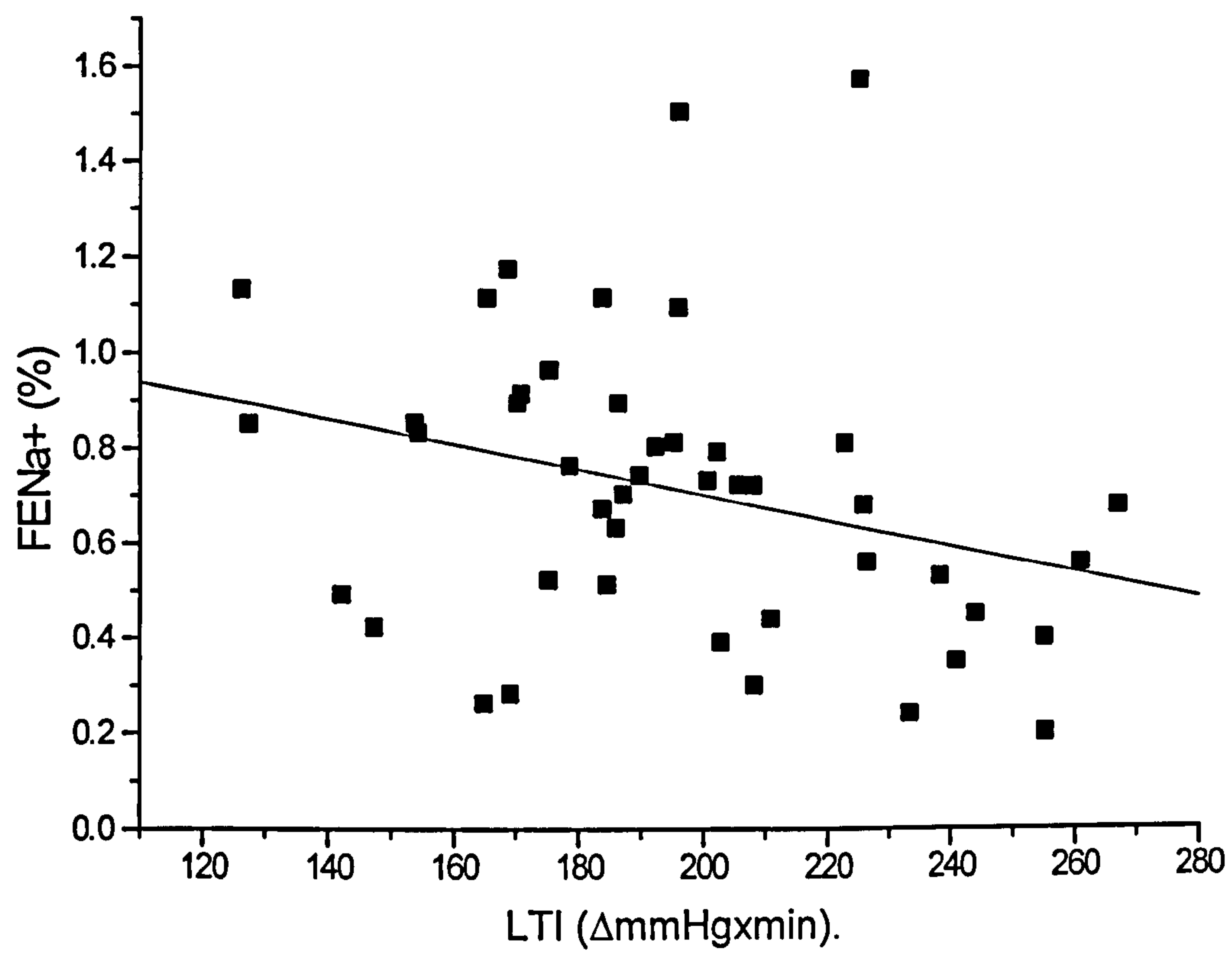


Figure 3.2.2 Relationship between fractional excretion of sodium and LTI ($r = -0.29$, $P < 0.05$).

Table 3.2.1. Daily control and response values for plasma volume (PV), blood pressure (SBP and DBP), heart rate (HR) and leg volume (LV). Peak = maximum value during LBNP; Δ = Peak value minus control value. Values are average ± SD. * = P < 0.05 compared to test day 1.

	Test day				
	1	2	3	4	5
% change in PV	-	-3.9±5.3	-3.9±9.1	-1.5±9.7	-3.3±10.8
LV (L)	9.9±1.1	9.9±1.1	10.0±1.1	10.0±0.9	9.9±1.0
Control SBP (mmHg)	113.7±13.1	114.0±10.8	112.6±11.0	111.1±9.6	111.4±8.8
Control DBP (mmHg)	70.8±9.9	68.1±9.0	67.1±10.5	65.7±7.8	65.6±10.0
ΔSBP (mmHg)	-21.0±9.0	-18.3±11.6	-19.2±6.9)	-17.6±7.9	-15.1 ±11.7
ΔDBP (mmHg)	-11.9±11.4	-5.0±10.5	-10.6±6.7	-11.4±5.3	-3.9±6.6
Control HR (b·min ⁻¹)	59.4±10.2	62.0±12.6	61.6±10.4	62.5±11.8	64.4±10.9
ΔHR (b·min ⁻¹)	56.7±14.1	60.1±18.9	65.2±18.5	61.8±15.6	62.4±19.9*
Peak HR (b·min ⁻¹)	116.1±16.8	122.7±17.9	126.8±19.3	124.3±18.9	126.8±17.8

3.2.4 Discussion.

In this study subjects were exposed to LBNP until the onset of pre-syncopal symptoms for five consecutive days. There were no changes in orthostatic tolerance and there was no evidence of changes in plasma volume or its regulation indicated by changes in haematocrit, haemoglobin, plasma protein concentration, or fractional excretion of sodium, 24 hours after each orthostatic stress test.

Control and orthostatic stress response values for blood pressure (SBP and DBP) and heart rate (HR).

Neither control blood pressure, or blood pressure responses to orthostatic stress changed during the 5 day test period (table 3.2.1; $P>0.05$). These data are in agreement with a previous study that exposed subjects to orthostatic stress repeatedly and reported no changes in blood pressure at rest or during orthostatic stress (Lightfoot et al, 1989). Further, there were no differences in peak heart rates (HR) or changes in HR during orthostatic stress (resting to peak HR; table 3.2.1; $P>0.05$) as previously reported. (Lightfoot et al, 1989). Therefore, from this and previously reported data (Lightfoot et al, 1989), it would appear that the consistency of HR and BP responses to repeated orthostatic stress do not explain differences between studies. Particularly since previous studies have shown increases in orthostatic tolerance following repeated exposure to LBNP (Hilton et al, 1988; Lightfoot et al, 1989) and the present study that showed no change in orthostatic tolerance. However, there was a significant increase in resting HR, as measured prior to orthostatic stress (table 3.2.1; $P<0.05$). Lightfoot et al (1989) reported a

similar change in resting HR during their study involving daily exposure to orthostatic stress, although the increase was not significant. From the present data it is difficult to speculate on possible mechanisms that may be responsible for this increase in resting HR. Since important humoral responses to orthostatic stress have been shown previously to return to baseline following termination of orthostatic stress (Laszlo et al, 2001), it would appear unlikely that these changes would have any residual effect on resting HR. A further possible cause of the resting HR increase could have been a possible alteration in sympatho-vagal balance related to repeated exposure to the vaso-vagal reflex, a common pre-syncopal symptom (Van Lieshout et al, 1991; Deitz et al, 1997). However, this response did not occur in all subjects following each orthostatic stress test. Analysis of the present data did not reveal a relationship between the number of vaso-vagal reflex responses immediately before LBNP test termination and the magnitude of resting HR increase.

Perhaps a more likely candidate could be a residual reduction in peripheral vascular resistance, leading to an increase in resting HR in order to maintain resting BP. Following exposure to orthostatic stress peripheral vasodilation has been reported previously from data collected using forearm occlusion plethysmography (Van Lieshout et al, 1991; Dietz et al, 1997). It is possible that a small residual peripheral vasodilatation occurred following repeated exposure to orthostatic stress, thus reducing peripheral vascular resistance.

Orthostatic tolerance (LTI) and fractional excretion of sodium (FE_{Na+}).

Previous studies have demonstrated that orthostatic tolerance increased when orthostatic stress was presented to subjects daily (Hilton et al, 1988; Lightfoot et al, 1989). Lightfoot et al. (1991) reported that adaptive increases in orthostatic tolerance could be avoided by separating LBNP tests by at least 72 hours. However, data in 3.1 showed that increased orthostatic tolerance occurred with 72 – 120 hours between orthostatic stress tests. Therefore, from this data it appears reasonable to assume that an increase in orthostatic tolerance could be expected with daily exposures to orthostatic stress, thus providing an opportunity to examine the possible mechanisms responsible for adaptive increases to orthostatic tolerance previously shown (Hilton et al, 1988; Lightfoot et al, 1989). The study in 3.1 suggested that perhaps 3-5 days between orthostatic stress tests was not sufficient to prevent adaptive increases in tolerance. Yet, the lack of an increase in orthostatic tolerance seen in the present study (figure 3.2.1; $P < 0.05$), when tests were performed daily, suggests that the length of time between tests does not explain the improvements in tolerance reported previously (Hilton et al, 1988; Lightfoot et al, 1989; also see 3.1).

One possible factor relating to the discrepancy between this and other studies (Hilton et al, 1988; Lightfoot et al, 1989) is the use of different indices for quantifying orthostatic tolerance. The studies of Hilton et al (1988) and Lightfoot et al (1989) reported the cumulative stress index (CSI; Luft et al, 1976), which has been shown to magnify changes in orthostatic tolerance (Lightfoot and Tsintgaris, 1995). The LBNP tolerance index (LTI), used in this study, has been shown to provide a more reliable estimate of changes in orthostatic tolerance (Lightfoot and Tsintgaris, 1995; see 3.1).

However, upon inspection of figure 2 of the report of Lightfoot et al (1989), it would appear that only three of the six subjects showed a noticeable increase in orthostatic tolerance, with one of these subjects showing a substantial increase when comparing day 1 with day 9. A similar finding was reported in 3.1 where there was an increase in average LTI, but within the group of twenty subjects, two showed increases in LTI that were noticeably greater than the rest of the group. Therefore, it is possible that some subjects possess greater susceptibility to increases in orthostatic tolerance when this stress is repeated.

Studies that have examined changes in Na^+ excretion during a single stage of LBNP have reported substantial reductions (Gilbert et al, 1966; Mauran et al, 1998). Further, Gilbert et al. (1966) showed that Na^+ excretion did not quite reach pre-orthostatic stress levels after an hour of post orthostatic stress supine rest, which may have a cumulative effect when orthostatic stress is presented repeatedly. Additionally, in the studies of Gilbert et al. (1966) and Muaran et al. (1998) only a single sub-tolerance, LBNP stage was used. It has been shown in the preceding study that responses to a given level of orthostatic stress may be related to individual orthostatic tolerance (see 3.1). Therefore, it was necessary to expose subjects to orthostatic stress until presyncope, in order to present a similar level of stress to each subject, during which responses in Na^+ excretion were examined.

However, no changes in FE_{Na^+} were observed during the 5 day test period (figure 3.2.1; $P < 0.05$). These measures were taken immediately prior to each LBNP test, and therefore were 24 hours after the previous exposure to LBNP. Whilst

speculative, this suggests that any changes in FE_{Na^+} , in response to LBNP induced disturbances in plasma volume, and occurred within the 24 hours between tests. From the present data, mechanisms responsible for previously reported increases in orthostatic tolerance, when orthostatic stress was present every 24 hours (Hilton et al, 1988; Lightfoot et al, 1989), are unlikely to be associated with changes in sodium excretion.

By way of further explanation, in one subject, a urine sample was collected within 10 minutes after each orthostatic stress test and this sample together with the venous blood sample collected immediately prior to each orthostatic stress test was used to calculate post orthostatic stress FE_{Na^+} . On average there was a 46% increase in FE_{Na^+} after orthostatic stress compared with immediately before. This large increase in FE_{Na^+} could have been a response, not to a reduction in plasma volume during orthostatic stress (Hagan et al, 1978; Noddeland et al, 1981; Lundvall and Bjerhoel, 1995; Brown et al, 1999) but to a post orthostatic stress increase in central blood volume, via the Henry-Gauer reflex (Gauer and Henry, 1976). Orthostatic stress related increases in central blood volume occur immediately after the negative pressure is reversed, and pooled blood is returned to the central circulation (Wolthuis et al, 1975). Furthermore, Boening et al (1972) demonstrated that rapid increases in Na^+ excretion could occur from the onset of water immersion, which is also known to increase central blood volume. Therefore, with such rapid responses in FE_{Na^+} to changes in central blood volume, the data from this one subject immediately after orthostatic stress and the data from all subjects 24 hours after orthostatic stress

suggests that changes in FE_{Na^+} probably occurred within 24 hours of the orthostatic stress test, after which there was probably a return to baseline.

Indices of changes in plasma volume and lower limb volume.

Changes in plasma volume regulation (Lightfoot et al, 1989) and the formation of oedema in the lower limbs (Hilton et al, 1988) have been suggested as factors that may explain the reported increases in orthostatic tolerance upon daily exposure to LBNP. However, in the present study there were no differences in plasma volume estimated from Hct and Hb (Dill and Costill, 1974; table 3.2.1; $P>0.05$) or plasma protein concentrations (figure 3.2.1; $P>0.05$). Hct, Hb and plasma protein concentration have been shown to increase during orthostatic stress when capillary transmural pressure and filtration increase in the dependant limbs leading to losses in plasma water (Fawcett and Wynn, 1960; Hagan et al, 1978; Noddeland et al, 1981; Lundvall and Bjerkhoel, 1995). However, in the present study there were no changes in leg volume (table 3.2.1; $P>0.05$) as a consequence of repeated exposure to orthostatic stress. Therefore, it appears that any loss in plasma water into interstitium of the lower limbs during orthostatic stress, was reabsorbed into the cardiovascular system within the 24 hour period between tests. The role of possible changes in FE_{Na^+} following exposure to orthostatic stress may be important in this readjustment of fluid within the body. However, further work is required to examine this possible effect in more detail.

3.2.5 Conclusions.

The results of the present study suggest that daily exposure to a pre-syncopal orthostatic stress does not induce any change in fractional excretion of sodium (FE_{Na+}), as measured 24 hours after the previous orthostatic stress. However, data from one subject did provide suggestive evidence that acute changes in FE_{Na+} could occur during the transient post orthostatic stress central hypervolaemic state. The present data also suggests that daily exposure to a presyncopal orthostatic stress does not induce adaptations leading to increases in orthostatic stress. In addition to non-significant changes in FE_{Na+} and orthostatic tolerance, there were no changes in the indices of changes in plasma volume or lower limb volume. Further work is required to examine the time course of changes in FE_{Na+} within 24 hours after exposure to orthostatic stress.

Chapter 4.

Resting Blood Pressure and Orthostatic Tolerance in Humans: The Effect of Isometric Exercise Training.

Parts of this study have been published as abstracts and a manuscript of this study has been accepted for publication;

R. HOWDEN, J.T. LIGHTFOOT, S.J. BROWN AND I.L. SWAINE. (2002). Orthostatic tolerance and resting blood pressure in humans: the effect of isometric exercise training of the legs. *The Journal of Physiology*, 539P.

R. HOWDEN, J.T. LIGHTFOOT, S.J. BROWN AND I.L. SWAINE. (2002). Orthostatic tolerance and resting blood pressure in humans: the effect of isometric exercise training of the arms. *The Journal of Physiology*, 539P.

R. HOWDEN, J.T. LIGHTFOOT, S.J. BROWN AND I.L. SWAINE. (in the press). The effects of isometric exercise training on resting blood pressure and orthostatic tolerance in humans. *Experimental Physiology*.

4. Resting blood pressure and orthostatic tolerance: the effect of isometric exercise training.

4.1 Introduction.

As described in chapter 1, cardiovascular responses and factors affecting tolerance to orthostatic stress have been studied for many years (e.g. Johnson et al, 1974; Luft et al, 1976; Abboud et al, 1979). However, a full understanding of factors that influence the cardiovascular responses leading to syncope remains elusive. The main components of the cardiovascular responses to orthostatic stress studied previously have been; heart rate (Wahbha et al, 1989) and blood pressure responses (Stevens, 1966; Sather et al, 1986) and factors such as lower limb compliance (Luft et al, 1976; Stevens et al, 1992) and lower limb muscle tension (Coles et al, 1957; Smith, 1987). A more detailed discussion of these responses was given in chapter 1.

During orthostasis, the primary challenge to the cardiovascular system is to maintain blood pressure and thus adequate cerebral perfusion (Rowell, 1993). Blood pressure responses to lower body negative pressure (LBNP) are often characterised by a reduction in systolic blood pressure (Sather et al, 1986; Stevens, 1966) which often reaches a value of less than 80mmHg, and this has been established as a sign of pre-syncope. It has been suggested that a lower initial systolic blood pressure during orthostatic stress could result in early syncope (Stevens, 1966) and therefore, resting blood pressure could be an important factor in determining responses and tolerance to orthostatic stress.

Short duration isometric exercise training has been shown to lower resting systolic blood pressure by between 4 and 14 mmHg (Kiveloff and Huber, 1971; Ray and Carrasco, 2000; Wiley et al, 1992; Peters et al, 2001). However, since these studies did not assess changes in orthostatic tolerance, the affects of such training-induced blood pressure changes on orthostatic tolerance remain unclear. The majority of studies that have investigated the affects of isometric training on resting blood pressure have used the relatively small muscle mass of the upper body (unilateral handgrip exercise; Peters et al, 2001; Ray and Carrasco, 2000; Wiley et al, 1992). There are no reports of the effects of isometric training involving large muscle groups such as the legs.

Finally, there remains a possibility that adaptations may occur in the exercise-trained muscle, such as increases in muscle mass or resting muscle tension. These adaptations are known to be associated with changes in orthostatic tolerance (Luft et al, 1976; Smith et al, 1987; Convertino et al, 1988). However, this appears to be evident only when the muscle group being trained is the same as that which is directly exposed to orthostatic stress (i.e. the legs). Therefore, it is necessary to compare orthostatic tolerance and resting blood pressure in exercise-trained limbs that are exposed to orthostatic stress, with exercise-trained limbs that are not. No previous studies have assessed the effects of isometric exercise training of both the arms and the legs, upon resting blood pressure and orthostatic tolerance.

Therefore, the purpose of this study was to investigate the effects of isometric exercise training of the arms and the legs upon resting blood pressure and orthostatic tolerance with account being taken of possible changes in muscle volume.

4.2 Methods.

Subjects

Twenty seven subjects completed the study and after being allocated to either 'training' or 'control' groups. Of the eleven subjects initially recruited to the exercise training programme, nine completed the first phase (training of the legs; T1) and eight completed the second phase (training of the arms; T2). Six subjects completed the entire training study (18 weeks – including the 5 weeks each of leg- and arm-training and intervening 8 week period). Two groups of eight subjects were allocated to the control groups (leg- or arm-training control period; C1 and C2 respectively). All subjects volunteered to participate in this study and were asked to complete a 'medical history' screening questionnaire. Each subject provided written informed consent prior to participation in the study and they were given a verbal and written description of all experimental procedures. The study was approved through the De Montfort University 'Procedures for Ethical Approval of Research'.

Nine subjects completed the isometric exercise training of the legs (T1; 7 males and 2 females; average \pm SD; resting SBP 121 ± 9 mmHg; resting DBP 70 ± 7 mmHg; age 21.1 ± 1.2 years; height 178.4 ± 7.5 cm; mass 78.6 ± 10.2 Kg) and eight subjects completed the isometric exercise training of the arms (T2; 6 males and 2 females; resting SBP 114 ± 11 mmHg; resting DBP 65 ± 5 mmHg; age 21.0 ± 1.4 years; height 177.1 ± 9.9 cm; mass 78.3 ± 10.3 Kg).

Resting blood pressure and orthostatic tolerance (LTI) were not different from pre-training values (after the 5 week leg-training programme and subsequent 8 week intervening period) when subjects commenced the isometric arm-training phase.

Two groups of eight subjects acted as controls (C1 and C2; 11 males and 5 females; resting SBP 118 ± 8 mmHg; resting DBP 71 ± 6 mmHg; age 24.5 ± 6.1 years, height 177.9 ± 9.9 cm; mass 75.8 ± 7.0 Kg). They completed all aspects of the study except for the isometric exercise training.

4.2.1 Equipment.

Orthostatic stress tests.

The LBNP chamber used in this study, the LBNP test procedure and methods of data collection have been described in detail elsewhere in chapter 2, sections 2.1 to 2.5. Briefly, subjects placed their lower extremities' inside the LBNP chamber and were then sealed at the level of the iliac crests. Internal chamber pressure was reduced sequentially until the onset of established presyncopal signs and/or symptoms. Following an initial orientation LBNP exposure to -40 mmHg, all subjects completed an orthostatic stress test before (LBNP₁), and after (LBNP₂) the exercise training period. Subjects reported to the laboratory for orthostatic stress testing after abstaining from exercise and consumption of alcohol for 24 hours and 2 hours post-prandial. Subjects were required to maintain their usual diet and level of physical activity during the course of the study.

Blood pressure (BP), electrocardiography (ECG) and electromyography (EMG).

All measures of BP were taken with an automated blood pressure recording device (TM-2541R, AND Instruments, UK) using the oscillometric technique and a pneumatic cuff placed around subjects' upper left arm. A full description of this technique and its verification is given in chapter 2, section 2.7. BP was measured and recorded during the last 20 seconds of each minute during all orthostatic stress tests. Reported values for resting blood pressure were the average of at least five measures of BP, recorded in all subjects following each week of the five-week isometric exercise training programmes. These measures of BP were taken before any isometric exercise was performed on the day of that measurement.

During all orthostatic stress tests surface ECG and EMG recordings were made from a standard three-lead configuration and the right vastus medialis muscle respectively. Heart rate was calculated continuously, by expressing each recorded R-R interval as beats per minute. The EMG data were recorded for two purposes. Firstly, EMG signals were used during orthostatic stress to ensure that lower limb muscle tension did not increase, thus aiding venous return. Secondly, an integrated signal was calculated from the EMG data by the data acquisition software (Chart v 4.0.4; AD Instruments, Hastings, UK) and used as an indicant of resting muscle tension. These procedures are described in full in chapter 2, sections 2.6.1 and 2.6.2.

Estimated lean leg volume (LLV).

Before and after isometric exercise training of the legs, lean limb volume was estimated using the anthropometric method of Jones and Pearson (1969). Briefly,

limb circumference was determined at seven sites, partitioning the leg into six truncated cones. Measures of skin fold thickness from four leg sites were also recorded to enable calculation of fat free limb volume. Full details of the method used to determine leg volume is provided in chapter 2, section 2.14 and figure 2.6.

4.2.2 Isometric exercise training.

All isometric exercise training was performed using an isokinetic dynamometer (Kin-Com, Chattanooga Group Inc., USA), with subjects suitably restrained (see 2.15). Isometric exercise of the arms was performed by contracting the elbow flexors at an elbow joint angle of 90°. Isometric exercise of the legs was performed by contracting the knee extensors at a knee joint angle of 120° (see 2.15).

At the start of each session subjects were required to perform at least two (a maximum of five) maximum voluntary contractions (MVC), separated by one minute of rest, that were not different by more than 20%. The maximum force produced from these two MVCs was used to calculate the isometric exercise intensity for that session.

The isometric exercise training protocol consisted of four, two-minute bouts of isometric exercise which were performed by subjects at either 30% (arm training) or 20% (leg training) of MVC. Each two-minute bout of exercise was separated by three minutes of seated rest (see 2.15). Sessions were repeated three times per week for five weeks. Subjects failing to attend more than two consecutive sessions or missed

more than three sessions were excluded from the study. This condition resulted in two subjects being excluded from the arm training and control groups. Two subjects from the leg training group were also excluded due to illness.

4.2.3 Overall study design.

A graphical representation of the overall study design is given in Figure 4.1. Subjects were allocated to either 'training' or 'control' groups for the duration of the study (18 weeks in total). The 'training' group first attended for isometric exercise training of the legs on three occasions per week for 5 weeks. Before commencing this training programme, and within 10 days of its completion, all subjects were assessed for resting blood pressure and orthostatic tolerance. After completion of the 5 week leg-training programme all subjects returned to their 'normal daily activities' for 8 weeks. After this intervening period subjects attended for isometric exercise training of the arms. They attended on three occasions per week for 5 weeks. Again, they were assessed for orthostatic tolerance and resting blood pressure at the commencement and at the completion of this training programme. The control group was asked to continue with their 'normal daily activities' for the duration of the study. They were asked to attend the laboratory for orthostatic stress tests and resting blood pressure measurement on similar occasions to the 'training' group subjects.

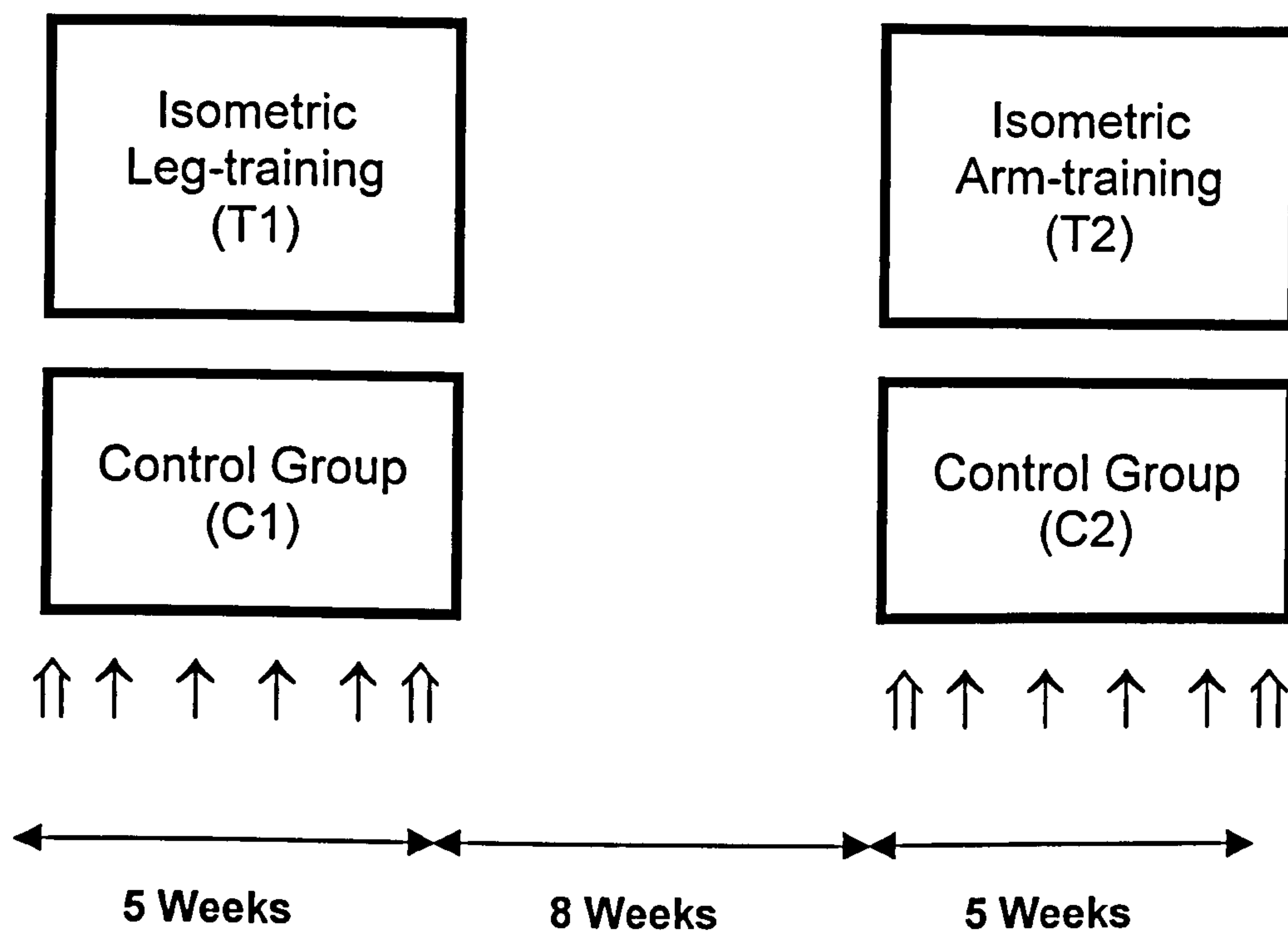


Figure 4.1 Schematic representation of the overall study design for assessment of the effects of isometric exercise training upon resting blood pressure and orthostatic tolerance (↑↑ indicates LBNP test and resting blood pressure measure; ↑ indicates resting blood pressure measure).

4.2.4 Statistical Analysis.

Comparisons between group averages for SBP and DBP before exercise training, during exercise training and immediately before the second LBNP test and groups were calculated using a one-way repeated measures analysis of variance (ANOVA). P values less than 0.05 were considered to show a statistically significant difference between given variable averages. From data that produced statistically significant results, the Student-Newman-Keuls test was used to identify the significance of specific differences. This test first compares the largest and smallest means and if significant then performs comparisons between the remaining means of the data analysed. Differences between group averages in the magnitude of blood pressure reduction (leg condition vs arm condition), LTI, responses to LBNP and the resting blood pressures and LTIs prior to each exercise training period of the subjects who completed both conditions, were assessed using a 2x4 ANOVA. P values of less than 0.05 were considered to show a significant difference between the averages and the location of these differences were assessed using student's t-tests following an adjustment to the alpha level using Bonferroni. Significant relationships between changes in LTI and differences in the haemodynamic responses to LBNP were reported and assessed using Pearson's correlation coefficient (r).

4.3 Results.

4.3.1 Isometric exercise training.

MVC

Five weeks of isometric exercise training resulted in all subjects showing an improvement in MVC. Significant increases in the average MVC produced in the final week of exercise training, compared with the average MVC produced in the first week of exercise training, were apparent (average \pm SD 603.4 \pm 219.9 vs 1024.8 \pm 127.8; T1 (legs) N and 257.4 \pm 56.4 vs 345.1 \pm 96.9; T2 (arms) N; $P>0.05$; figure 4.2).

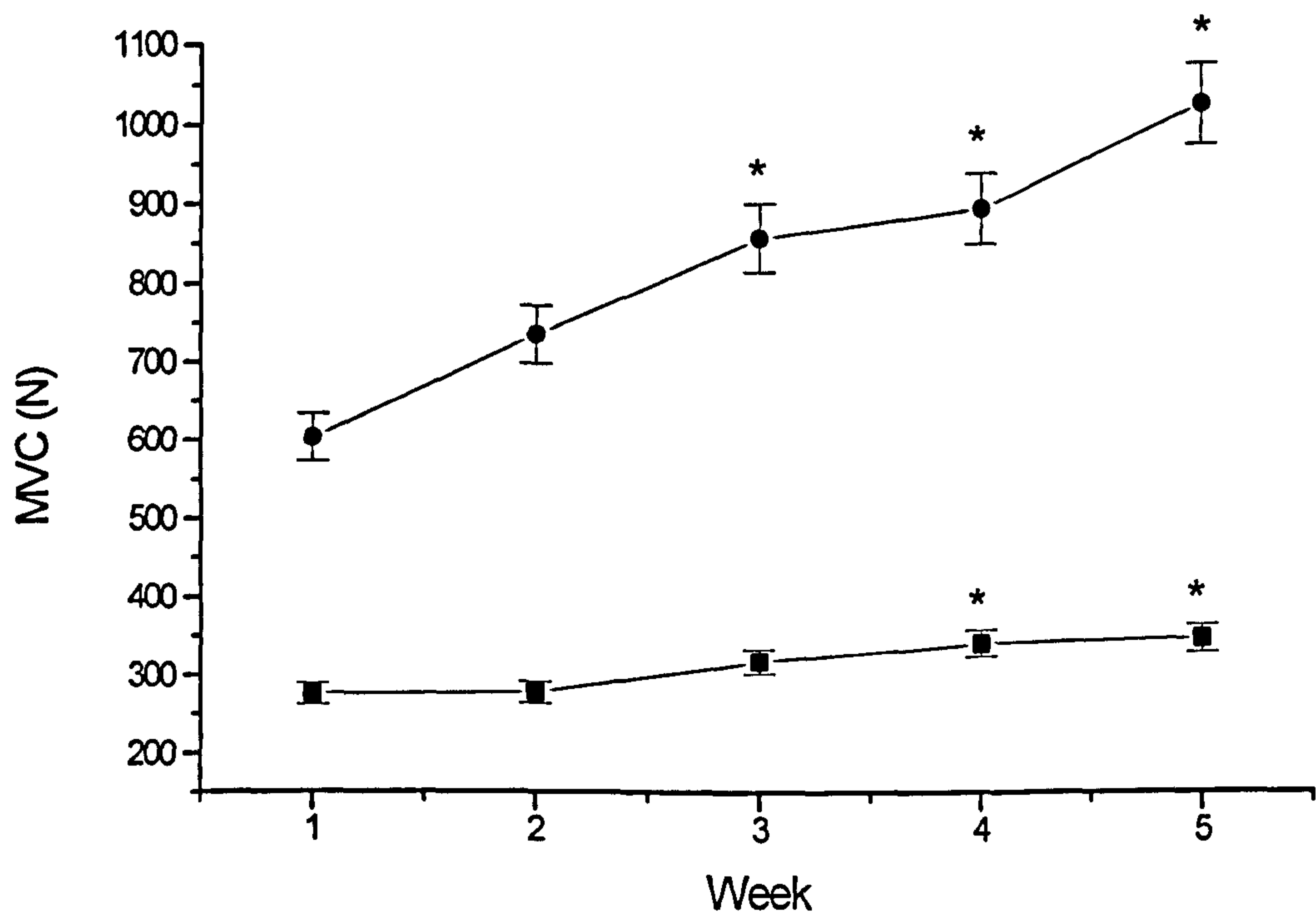


Figure 4.2. Average \pm SD Maximum Voluntary Contraction (MVC) during 5 weeks of isometric training of the legs (●) and arms (■). * = significant increase when compared to week 1.

Resting blood pressure.

Resting SBP was reduced in both the leg (T1) and arm conditions (T2) following isometric exercise training (120.7 ± 9.6 vs 110.7 ± 8.4 ; T1 and 114.3 ± 11.3 vs 101.9 ± 7.7 ; T2 mmHg; $P < 0.05$; figure 4.3). The magnitude of SBP change was not different when comparing the leg condition with the arm condition (10.0 ± 8.7 vs 12.4 ± 9.3 mmHg; $P > 0.05$). However, despite conducting all LBNP₂ tests four to ten days after the completion of the isometric exercise training, resting SBP was not significantly different immediately before the LBNP₂ test when compared to resting SBP before isometric exercise training in both T1 and T2 (120.7 ± 9.6 vs 115.6 ± 12.1 and 114.3 ± 11.3 vs 117.0 ± 12.3 mmHg respectively; $P > 0.05$; figure 4.3). SBP in the control groups (C1 and C2) did not change during the five-week exercise training period and was not different immediately prior to the LBNP₂ test (118.0 ± 8.6 vs 119.6 ± 6.1 vs 118.3 ± 7.8 for C1; 116.9 ± 9.6 vs 115.2 ± 12.2 vs 114.0 ± 10.1 mmHg for C2; $P > 0.05$). The SBP of the six subjects who completed both exercise training conditions was not significantly different when comparing SBP prior to the arm exercise training condition with SBP prior to the leg exercise training condition (123.3 ± 7.4 vs 119.5 ± 2.8 mmHg; $P > 0.05$).

DBP did not change significantly during the isometric exercise training period in T1, T2, C1 or C2 compared to pre-training DBP (70.3 ± 7.4 vs 66.7 ± 11.2 for T1; 64.8 ± 5.6 vs 58.8 ± 12.3 for C1; 70.8 ± 6.4 vs 72.8 ± 9.4 for T2; 71.2 ± 8.3 vs 72.2 ± 7.3 mmHg for C2; $P > 0.05$; figure 4.3).

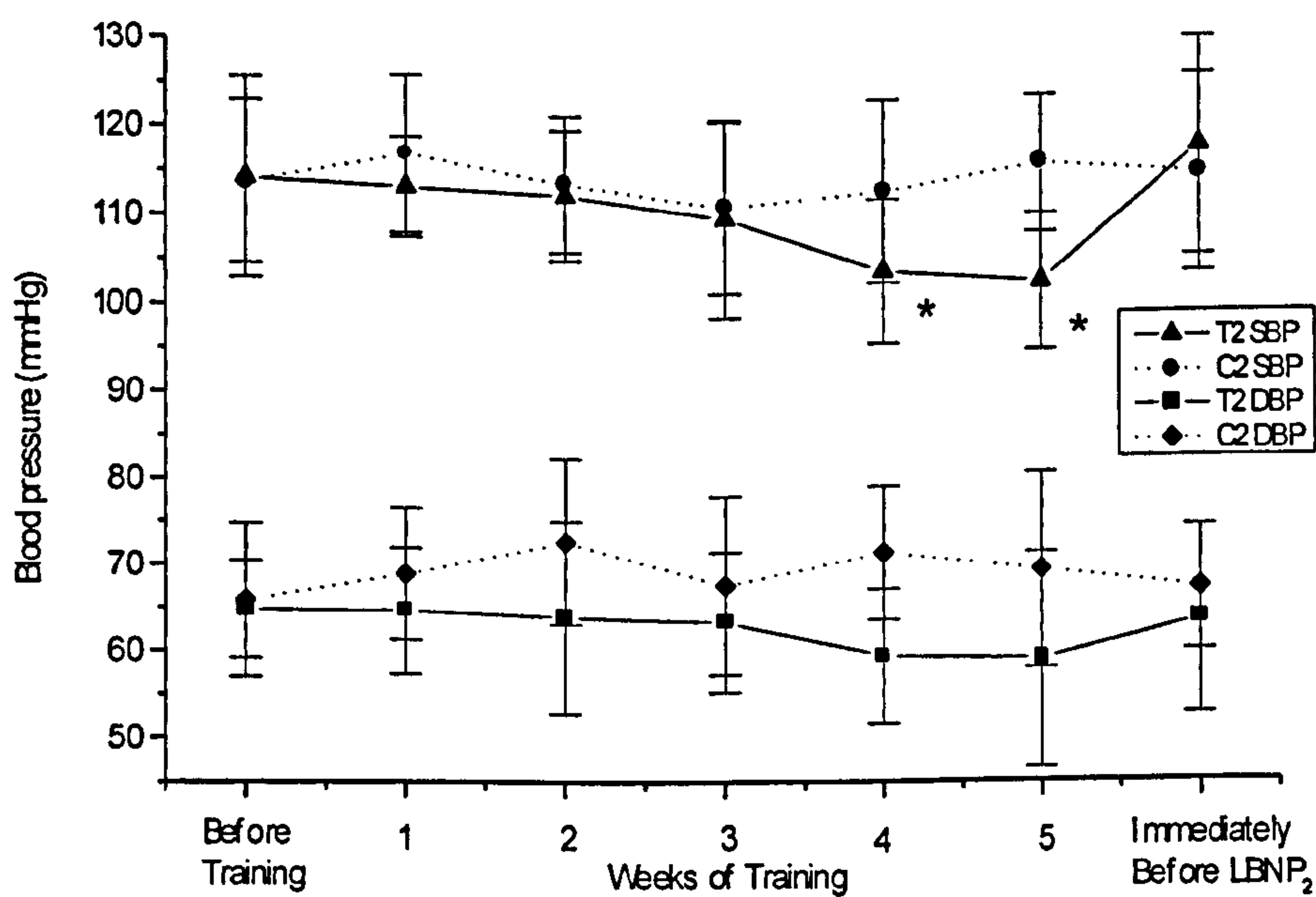
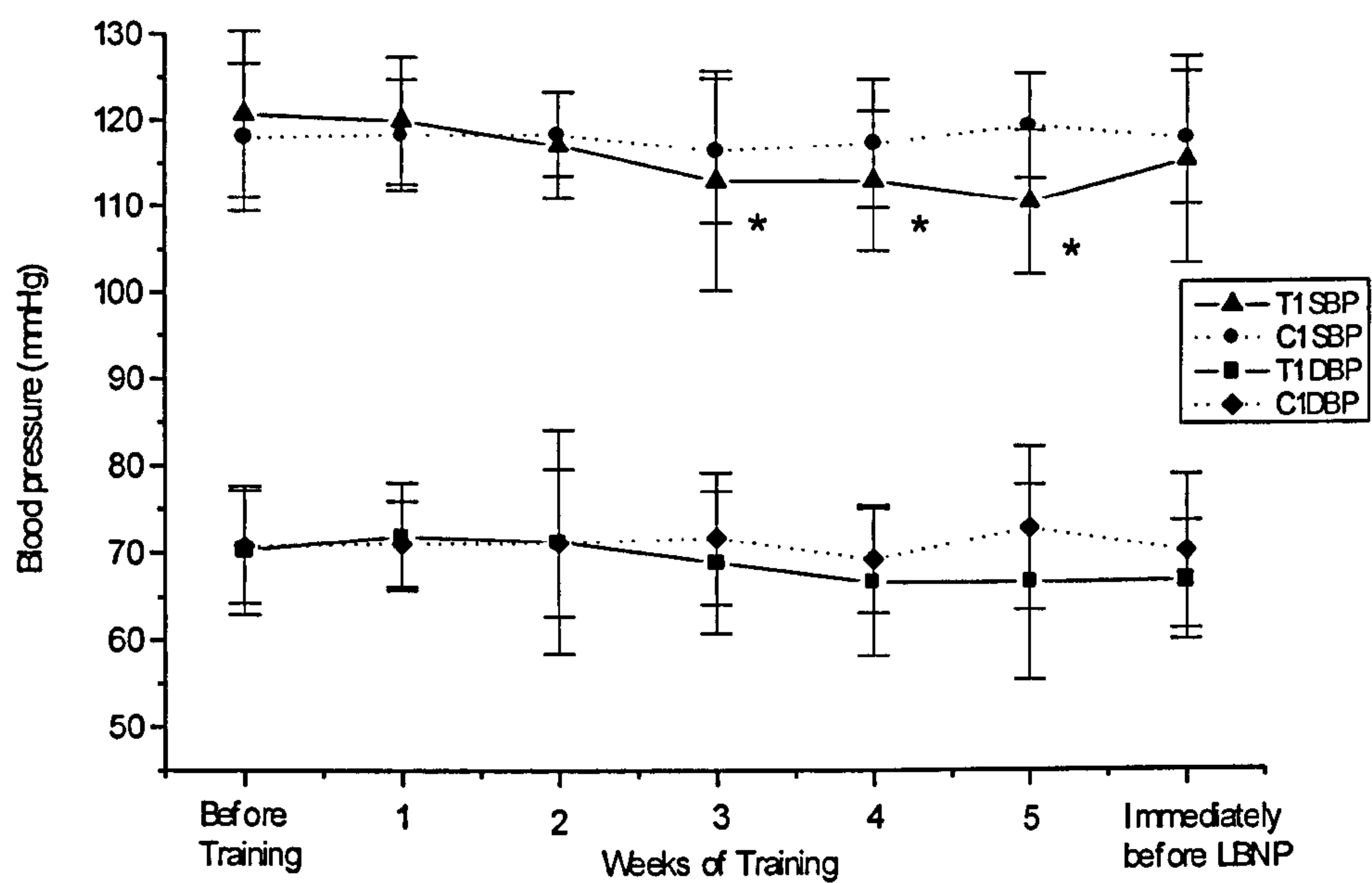


Figure 4.3. Average \pm SD resting systolic (SBP) and diastolic (DBP) blood pressure, before training, during 5 weeks of training and immediately before the final orthostatic stress test for leg-training (T1 training group; C1 control group; top panel) and arm-training (T2 training group; C2 control group; bottom panel). * = significant reduction when compared to 'before training'.

4.3.2 Orthostatic tolerance.

LTI.

There was a significant increase in LTI in the T1 group when comparing LBNP₁ with LBNP₂ (226.2 ± 40.9 vs 247.0 ± 39.5 LTI; $P < 0.05$). There was not a significant difference in LTI between LBNP₁ and LBNP₂ in groups T2, C1 or C2 (231.9 ± 55.6 vs 212.8 ± 34.9 for T2; 217.5 ± 37.3 vs 211.1 ± 30.9 for C1; 218.4 ± 35.4 vs 216.6 ± 32.5 LTI for C2; $P > 0.05$; figure 4.4). Also the pre-exercise training LTIs were not different when comparing the orthostatic tolerance of the subjects who completed both leg and arm exercise training (234.8 ± 63.0 vs 235.8 ± 33.6 LTI; $P > 0.05$).

Responses to orthostatic stress.

There were no differences in the change (Δ) in HR, SBP, or DBP during LBNP₁ or LBNP₂ in groups T1, T2, C1 or C2 (Table 4.1; $P > 0.05$). The time to peak HR was significantly increased during the orthostatic stress test, which followed leg training (Table 4.1; $P < 0.05$). The time peak HR was not significantly different between LBNP tests in the T2 and C groups (Table 4.1; $P > 0.05$). The difference in time to peak HR between LBNP₁ and LBNP₂ were calculated in addition to the difference in LTI between the two orthostatic stress tests. These values were then correlated (Figure 4.5) producing a significant relationship ($r = 0.84$; $P < 0.05$). Figure 4.5 includes data from both the T and C groups.

The slope of the integrated EMG signal was calculated during the final minute of the control period immediately before orthostatic stress. There were no significant

changes in the integrated EMG slope in LBNP₂ compared to LBNP₁ (Table 4.1; $P>0.05$). These measures were not made in the T2 or C2 group since there was no rationale for increases in resting muscle tone in the legs following isometric exercise training of the arms.

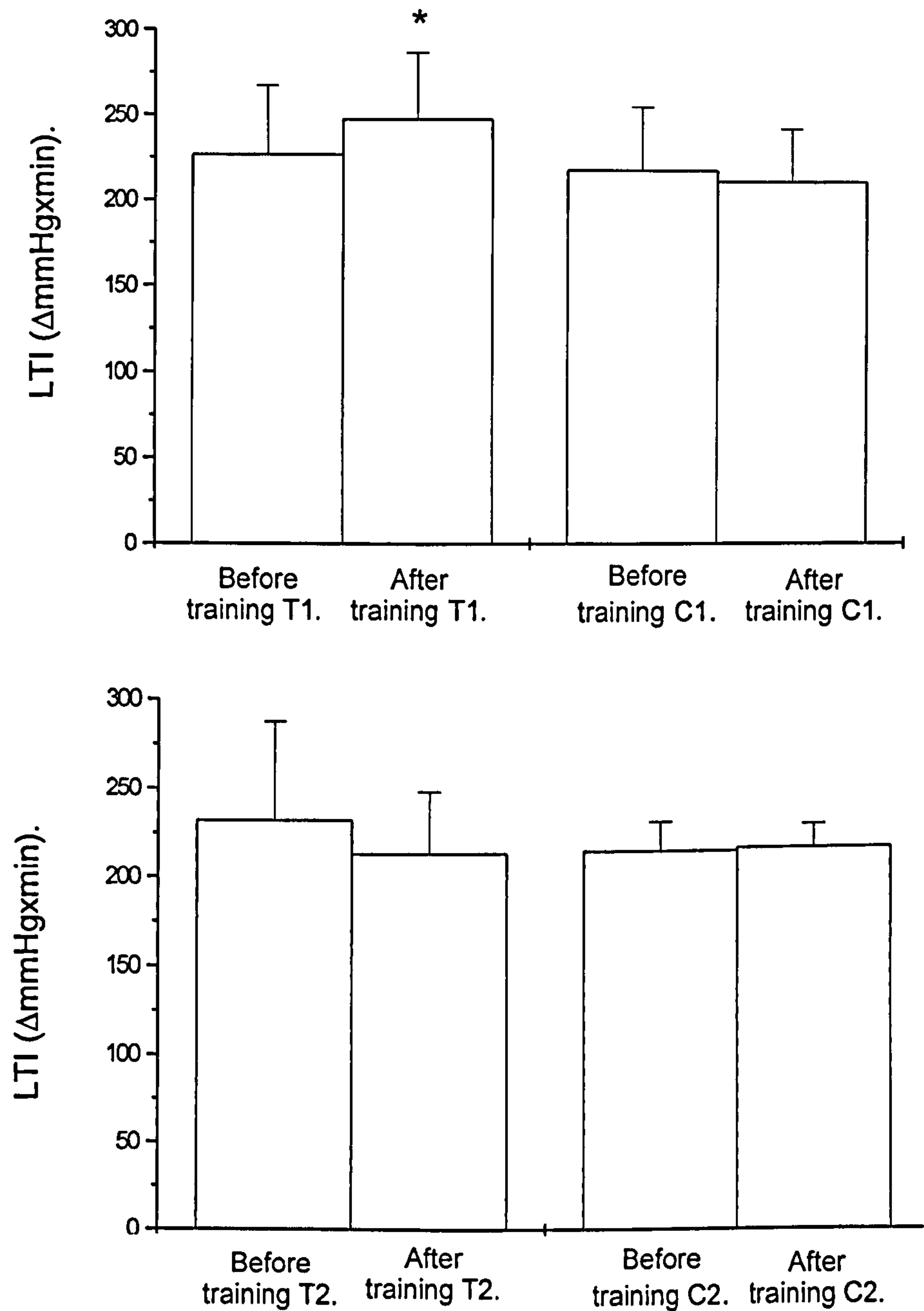


Figure 4.4. Group average \pm SD LBNP tolerance index (LTI) before and after training of the legs (top panel; T1 and C1) and arms (bottom panel; T2 and C2). * = significant increase when compared to 'before training'.

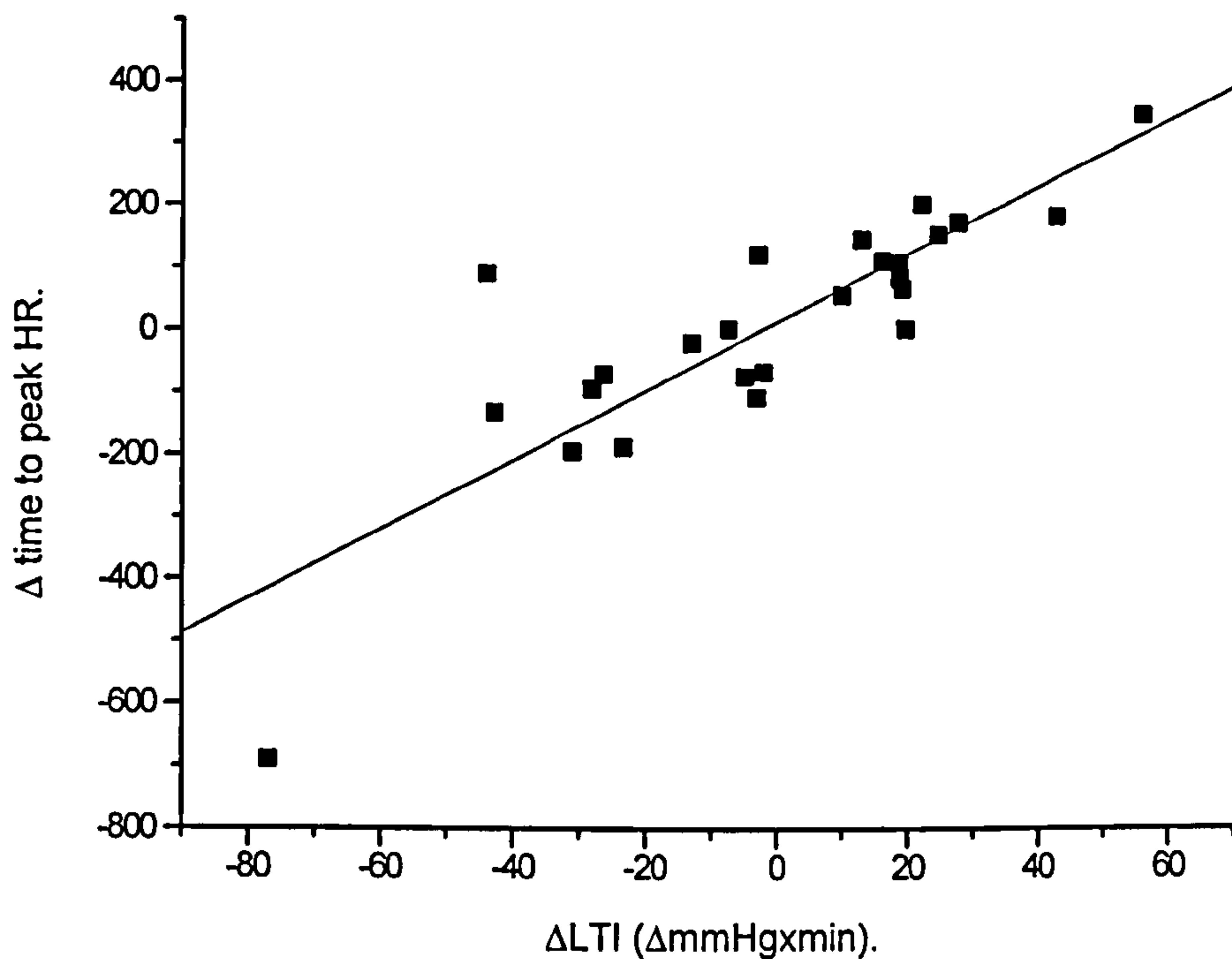


Figure 4.5. Relationship between the difference in time to peak HR during orthostatic stress between LBNP₁ and LBNP₂ and changes in LTI from LBNP₁ to LBNP₂. ($r = 0.84$; $P < 0.05$).

Estimated lean leg volume.

There was a small, but significant, increase in lean leg volume following isometric exercise training of the legs ($P < 0.05$; table 4.1). This increase in lean leg volume was not apparent in the C1 group ($P > 0.05$; table 4.1).

Table 4.1. HR and BP responses during LBNP. Values are group averages \pm SD. Δ = baseline value minus greatest change during LBNP, excluding blood pressure and HR at pre-syncope. * = significant difference comparing LBNP₁ and LBNP₂ (P<0.05)

	GROUP	LBNP ₁	LBNP ₂
Time to peak HR (s)	T1	1462.4 \pm 257.2	1582.2 \pm 257.8*
	C1	1390.8 \pm 353.1	1390.6 \pm 196.4
	T2	1485.6 \pm 388.3	1383.9 \pm 199.2
	C2	1417.4 \pm 131.5	1385.9 \pm 41.4
Δ SBP during LBNP (mmHg)	T1	-20 \pm 18	-19 \pm 15
	C1	-20 \pm 12	-16 \pm 9
	T2	-14 \pm 12	-15 \pm 13
	C2	-19 \pm 14	-16 \pm 8
Δ DBP during LBNP (mmHg)	T1	-5.6 \pm 8.9	-11.0 \pm 15.6
	C1	-3.0 \pm 15.4	-6.0 \pm 14.0
	T2	-12.6 \pm 9.9	-6.8 \pm 7.3
	C2	-8 \pm 11	-6 \pm 11
Δ HR during LBNP (b \cdot min ⁻¹)	T1	64.4 \pm 18.8	70.9 \pm 50.9
	C1	48.1 \pm 16.2	52.5 \pm 15.6
	T2	57.5 \pm 16.1	57.4 \pm 13.2
	C2	50.6 \pm 15.4	47.4 \pm 13.4
Integrated EMG slope (mVs ⁻¹ s ⁻¹)	T1	0.01 \pm 0.02	0.01 \pm 0.01
	C1	0.01 \pm 0.02	0.01 \pm 0.02
	T2	•	•
	C2	•	•
Estimated lean leg volume (L)	T1	7.3 \pm 1.4	7.4 \pm 1.4
	C1	7.5 \pm 0.5	7.5 \pm 0.6
	T2	•	•
	C2	•	•

4.4 Discussion.

The aim of this study was to assess the effect of reduced resting blood pressure on orthostatic tolerance. In both the leg and arm isometric exercise training conditions, there was a significant reduction in resting SBP ($P < 0.05$) but not in DBP ($P > 0.05$). Orthostatic tolerance increased following leg isometric exercise training ($P < 0.05$), but was not different after arm exercise training ($P > 0.05$). There were no differences in the magnitude of changes, during or immediately before orthostatic stress, in HR, SBP or DBP before compared to after exercise training in either group ($P > 0.05$).

orthostatic tolerance and resting blood pressure.

The differences in the change in orthostatic tolerance between T1 and T2 suggest that the affect of reduced SBP on orthostatic tolerance was not consistent between the leg and arm isometric exercise training. Thus, since the reductions in SBP were accompanied by concomitant changes in orthostatic tolerance following leg training only, there remains a possibility that this observation was due to factors specific to isometric exercise training of the legs. Following isometric exercise training, changes in the musculature of the exercise trained limbs may have influenced the cardiovascular responses to orthostatic stress, if those limbs were directly exposed to the negative pressure (i.e. the legs). The increase in LLV noted in the leg training group may have been due to increases in muscle mass. A previous study has shown that lower limb venous compliance is negatively correlated with lower limb muscle cross sectional area (Convertino et al, 1988). This increase in muscle mass may decrease venous transmural pressure, thus attenuating venous distension during

increases in capillary hydrostatic pressure (e.g. LBNP; Convertino et al, 1988). A negative correlational relationship between lower limb compliance and orthostatic tolerance has also been demonstrated (Luft et al, 1976). Therefore, lower leg compliance may have attenuated the established reduction in venous return (Al-Shamma and Hainsworth, 1985; Hainsworth and Al-Shamma, 1988) thus influencing HR responses, during orthostatic stress. Support for this explanation of increased orthostatic tolerance noted in the present subjects was evident by the increase in the time to peak HR in T1 during the orthostatic stress test after leg training, which was not apparent after arm training. The difference in time to peak HR was also significantly correlated with the difference in LTI in this group ($r = 0.86$; $P < 0.05$). This relationship was also evident when these data from all subjects were included in the correlational analysis ($r = 0.84$; $P < 0.05$; figure 4.5).

However, possible changes in lower limb muscle tension following isometric exercise training of the legs may also partially explain the increases in LTI following leg training. Smith et al (1987) demonstrated an attenuated reduction in stroke volume and cardiac output during orthostatic stress with increased lower limb muscle tension. This altered response to orthostatic stress was attributed to compression of the peripheral vascular system thus maintaining venous return. However, in the present study calculation of the slope of the integrated EMG signal during the final minute of the control period immediately before orthostatic stress revealed no difference after isometric exercise training, thus indicating no change in resting muscle tension (table 4.1).

The increase in orthostatic tolerance in the leg training group could be related to the reduction in resting SBP. Conversely, the changes in the lower limb following leg training could have masked the effect of lower resting blood pressure on orthostatic tolerance. However, when a reduction in resting blood pressure was induced by isometric exercise training of the arms, this did not affect orthostatic tolerance. These findings suggest that the low orthostatic tolerance reported by Stevens (1966), was not attributable to a lower baseline SBP, but other factors, for example the absence of an increase in HR during orthostatic stress.

An important consideration when interpreting the results in the present study was the transient nature of the BP reductions (figure 4.3). The reductions in SBP in both the leg and arm isometric exercise training conditions were similar to those reported previously (Wiley et al, 1992). Wiley et al (1992) provided evidence to suggest that SBP could remain significantly reduced for up to fourteen days after completion of isometric exercise training. All post-training orthostatic stress tests in this study were conducted within ten days of completion of this exercise training and yet SBP, in both the leg and arm condition, was not different at this time compared to before isometric exercise training. However, it is possible that this recording of BP did not reflect resting SBP since the measurement was made immediately before orthostatic stress, a physiological stressor which was known to the subject.

The mechanisms associated with reductions in SBP following isometric exercise training and the dynamics of that change after training has ceased, remain unclear. Wiley et al (1992) suggested that the observed reductions in BP following isometric

exercise training might be associated with baroreceptor resetting after repeated exposure to the established pressor response to isometric contractions (Blomqvist, 1987; Hanson and Nagle, 1987; Longhurst and Stebbins, 1992). However, a resetting of baroreceptors to a lower operating point in response to the hypertensive stimulus of isometric exercise would be unlikely since, in hypertension, baroreceptors operate at a point that is proportional to the blood pressure stimulus and therefore reset to a higher pressure (Eckberg and Sleight, 1992). Furthermore, HR and BP immediately before LBNP were not different after isometric exercise training when compared to before training, suggesting that there was either no baroreceptor resetting or that any resetting was transient. Therefore, to fully assess the effect of a lower blood pressure on orthostatic tolerance it may be necessary to elicit a reduction in BP immediately before the orthostatic stress test.

4.6 Conclusions.

The isometric exercise training programme used in this study successfully reduced SBP in both the leg and arm conditions. Isometric leg exercise training resulted in a significant increase in orthostatic tolerance. It is possible that this improved orthostatic tolerance was associated with the significant increase in lean leg volume that may have contributed to improved venous return, indicated by the delay in the time to peak HR during the second orthostatic stress test (LBNP₂). This potentially masked the effect of reduced resting blood pressure on orthostatic tolerance.

However, isometric arm exercise training did not affect orthostatic tolerance, suggesting that there was no effect of a reduction in resting SBP. This provides further support for the notion that improved orthostatic tolerance following isometric exercise training of the legs was associated with local changes specific to the legs.

The reductions in SBP were not evident immediately before the orthostatic stress test after isometric exercise training of the legs or arms. This apparent return of SBP to the values recorded before isometric exercise training may have confounded the effect of reduced resting SBP on orthostatic tolerance.

Chapter 5.

Baroreflex and chemoreflex stimulation: Implications for tolerance to orthostatic stress.

The first study in this chapter was conducted at the Dept. of Kinesiology, University of North Carolina, Charlotte, USA following kind invitation from Prof. J.T. Lightfoot. A grant from the Dale and Rushton fund was awarded for travel expenses. The first study in this chapter has also been submitted as an abstract to the Physiological Society for publication in the proceedings of a forthcoming scientific meeting (University of Liverpool, 9-11th July, 2002),

HOWDEN, R., SWAINE, I.L., BROWN, S.J., LIGHTFOOT, J.T., TURNER, M.J.
(submitted) Responses of forearm blood flow to neck suction and neck pressure in humans.

5.1 Responses of the heart and forearm blood flow to application of suction and pressure to the neck.

5.1.1 Introduction.

As discussed in chapter 1, reflex changes in the flow of blood to the forearm have been attributed exclusively to cardiopulmonary baroreceptors during reductions in central venous pressure (CVP) (Abboud et al, 1979; Johnson et al, 1974; Lightfoot et al, 1989; Mack et al, 1993; Rowell, 1993). However, others have reported reflex changes in peripheral vascular resistance or blood flow during stimulation of carotid sinus baroreceptors (Wallin et al, 1975; Lindblad et al, 1982; Ebert, 1982; Victor and Mark, 1985; Duprez et al, 1987). Previous reports have demonstrated an increase in the sensitivity of the carotid sinus baroreflex with reductions in CVP (Victor and Mark, 1985; Pawelczyk and Raven, 1989; Potts et al, 1995) and a reduction in carotid sinus baroreflex sensitivity upon increases in CVP (Shi et al, 1993). This suggests an interaction between the cardiopulmonary baroreceptors and carotid sinus baroreceptor populations.

Mild levels of lower body negative pressure (LBNP), reducing CVP, and unloading the cardiopulmonary baroreceptors, have been shown to decrease forearm blood flow without an accompanying reduction in arterial blood pressure or an increase in heart rate (Johnson et al, 1974). However, it is possible that small reductions in CVP, and therefore stroke volume, may affect the pulsatile stimulus to arterial baroreceptors (Hainsworth, 1990). It has also been shown that reductions in arterial

blood pressure (Hisdal et al, 2001), and increases in heart rate (Thompson et al, 1991) are possible under mild orthostatic stress, suggesting that arterial baroreceptors may be partially responsible for the observed changes in forearm blood flow during reductions in CVP. Furthermore, stimulation of receptors in the pulmonary vein-atrial junction and right ventricle in dogs, has produced cardiovascular responses that are not in agreement with those associated with cardiopulmonary baroreceptor unloading in humans (Carswell et al, 1970; Crisp et al, 1988). Subsequently, it has been suggested that the concept of cardiopulmonary baroreceptors as a distinct population is not valid (Hainsworth, 1990). Therefore, it is possible that the control of forearm blood flow could be mediated by groups of baroreceptors other than those in the right ventricle and pulmonary vasculature.

Stimulation of the baroreceptors in the carotid sinus bifurcations may enable the investigation of the effect of the carotid sinus baroreflex on forearm blood flow. A common method for carotid sinus baroreceptor stimulation is the application of positive and negative pressure to the neck over the carotid sinus regions. A neck chamber device, developed for this purpose, was first described by Ernsting and Parry (1957), and further developed by Eckberg et al (1975) and Sprenkle et al (1986). Cardiovascular responses to neck suction are characterised by bradycardia and decreases in arterial blood pressure, while neck pressure results in increases in both heart rate and arterial blood pressure.

Investigations of the effect of a single neck suction stage on peripheral blood flow have been equivocal. Increases (Lindblad et al, 1982), decreases (Ebert, 1982; Victor and Mark, 1985; Duprez et al, 1987) and no change (Bevegård et al, 1977;

Thompson et al, 1991; Escourrou et al, 1993) in forearm or digital blood flow during carotid sinus baroreceptor stimulation have been reported. These different responses may be due to the use of multiple types of assessment protocols that do not assess the full stimulus-response curves for this baroreceptor region (Eckberg, 1980). From the position of the operating point on this function curve it is clear that a number of both positive and negative neck pressures are necessary to fully investigate carotid sinus baroreceptor influence on forearm blood flow. The majority of the studies in this area have reported the use of only one or two stages of neck suction and no application of neck pressure (Abboud et al, 1979; Bevegård et al, 1977; Ebert, 1982; Escourrou et al, 1993). Lindblad et al (1982) used a six second ramp of neck suction from -10 to -40 mmHg, whereas, Victor and Mark (1985) applied only one stage of neck pressure in their investigation of baroreflex control of vascular resistance. Given earlier work by Eckberg (1980), it appears that applications of pressure that do not cover the full baroreceptor operating range have presented a limited range of stimuli to the carotid sinus baroreceptors and this may explain the variability in previous reports.

The purpose of the present study was to assess the alterations in forearm blood flow in response to a wide range of positive and negative pressure changes in carotid sinus distending pressure.

5.1.2 Methods.

Subjects.

Twelve normotensive subjects [5 males and 7 females; average \pm SD, systolic blood pressure (SBP) 118 ± 14.9 mmHg; diastolic blood pressure (DBP) 63.8 ± 8.2 mmHg; age 23.4 ± 2.8 years; height 166.8 ± 9.9 cm; mass 68.0 ± 11.9 Kg] volunteered to participate in this study and were medically screened using a pre-participation questionnaire prior to testing. After receiving a verbal and written description of the experimental procedures, all subjects gave written informed consent to participate. All subjects were less than 30 years of age and had no history of any cardiovascular disorders. The University of North Carolina Charlotte Institutional Review Board approved all procedures. All subjects reported to the laboratory four hours post-prandial and were required to refrain from consumption of caffeine for four hours and participation in exercise for twelve hours prior to testing.

Forearm blood flow (FBF).

Measures of FBF were recorded using forearm occlusion plethysmography described elsewhere (Lightfoot et al, 1989). Briefly, a mercury-in-silastic strain gauge was placed on the right forearm at the point of maximum forearm circumference. A small pneumatic cuff placed around the right wrist of the subject was inflated to a suprasystolic pressure to exclude circulation to the hand during the measurement of FBF. A second pneumatic cuff, placed around the upper right arm, was inflated abruptly to 30-50 mmHg at the beginning of each measurement, thus occluding venous drainage from the arm. The rapid inflation was achieved by the use of a cylindrical accumulator controlled by solenoids. The triggering of the controlling

solenoids was synchronised with the neck suction/pressure stimulus. Each measurement of FBF was made during a standard 8 seconds of venous occlusion. However, to allow for a possible latency in sympathetic vasoconstrictor responses (Rea and Eckberg, 1987; Sugiyama et al, 1996) following the neck suction/pressure stimulus, the final 1 second of the 8 second FBF recorded signal slope was used to calculate FBF. FBF was calculated as $\text{mL} \cdot \text{min} \cdot \text{dL}^{-1}$ of forearm tissue, as described in detail in chapter 2, section 2.16.

Electrocardiography (ECG) and blood pressure (BP).

Surface ECG recordings were made using a standard three-lead electrode placement (Quinton 4500, Seattle, WA, USA) throughout the test period. Recordings of SBP and DBP were taken using a pneumatic cuff connected to an automated blood pressure device (Colin STBT – 680, San Antonio, TX, USA), which had been validated previously (Lightfoot et al, 1994). SBP was recorded immediately prior to the test period, during the interval when the neck chamber was changed from negative to positive pressure and after the third of the five negative pressure stages.

Carotid sinus baroreceptor stimulation.

Following a 10 minute period of supine rest and simultaneous subject instrumentation, the distending pressure of the carotid sinus was manipulated by application of neck suction (Eckberg et al, 1975) and neck pressure (Sprenkle et al, 1986; see 2.18). The neck chamber was positioned so that changes in ambient pressure were applied to tissues over the carotid sinus bifurcations. During an end expiration breath hold, five ECG R waves were recorded, after which an electronic solenoid was activated to initiate the pressure change within the neck chamber and

the simultaneous inflation of the upper arm venous occlusion cuff. The pressure change was sustained, while a further three ECG R waves were recorded, for a total of 8 seconds. Internal chamber pressure was then returned to ambient pressure and subjects were instructed to breath *ad libitum*. Nine chamber pressure levels were used and each pressure level was repeated five times with approximately 15 seconds between each stimulus. The chamber pressures, applied in sequence, were control (chamber pressure = ambient pressure), -12, -24, -36, -48, -60, 10, 20, and 40 mmHg.

Statistical analysis.

Differences in mean FBF and changes in R-R interval ($\Delta R-R$) between the different levels of neck stimuli were calculated using a one-way repeated measures ANOVA. P values less than 0.05 were considered to show a significant difference between FBF or $\Delta R-R$ means. When statistical differences were noted, Student-Newman-Keul's post hoc test was used to determine where differences lie. Relationships between chamber pressure and FBF, chamber pressure and $\Delta R-R$, and $\Delta R-R$ and FBF, were examined using the Pearson Correlation Coefficient. The stimulus-response characteristics of the carotid-cardiac baroreflex plot were examined by fitting a sigmoidal (Boltzman) function using the curve-fitting schedule of Microcal Origin (version 3.5).

5.1.3 Results.

Carotid sinus-cardiac baroreflex.

The effect of the changes in neck chamber pressure on R-R interval was defined as the carotid sinus/cardiac baroreflex (Eckberg et al, 1980). Plotting neck chamber pressure against $\Delta R-R$ (figure 5.1.1) demonstrated responses similar to those reported in previous reports (Hainsworth and Al-Shamma, 1988; Lightfoot et al, 1989; Levine et al, 1991).

The pre-stimulus R-R interval was calculated by averaging the two R-R intervals ($R-R_1$) immediately preceding the neck chamber stimulus. The second R-R interval during the neck stimulus ($R-R_2$) was calculated as the R-R interval response to neck suction/pressure. Subsequently the change in R-R interval ($\Delta R-R$) from pre-stimulus to that of during the stimulus was derived from subtracting $R-R_2$ from $R-R_1$. Since each neck suction/pressure was repeated five times, the five $\Delta R-R$'s from each neck stimulus were averaged to produce a mean $\Delta R-R$ for each chamber pressure applied.

FBF and $\Delta R-R$ responses to neck suction/pressure.

There was no significant change in FBF when the carotid sinus baroreceptors were stimulated by four stages of neck suction at -12, -24, -48 and -60 mmHg (2.68 ± 0.77 vs 2.60 ± 0.69 vs 2.69 ± 0.69 vs 2.54 ± 0.82 vs 2.68 ± 0.89 mL·dL·min⁻¹, respectively) when compared to control (2.57 ± 0.61 mL·dL·min⁻¹; $P < 0.05$). FBF, during application of neck pressures of 10, 20, and 40 mmHg (2.74 ± 1.55 vs 2.66 ± 0.71 vs

$2.43 \pm 0.6 \text{ mL}\cdot\text{dL}\cdot\text{min}^{-1}$, respectively), was also not significantly different from control ($P>0.05$; figure 5.1.2). Application of neck suction induced significant increases in $\Delta\text{R-R}$ at all stages (71.56 ± 93.52 vs 130.47 ± 103.68 vs 176.72 ± 105.56 vs 218.39 ± 101.8 vs 253.75 ± 114.71 ms, respectively; $P<0.05$; figure 2). Neck pressure produced a small reduction in R-R interval (e.g. -24.7 ± 40.29 ms at +20mmHg and -48 ± 48.15 ms at +40 mmHg) that was not significantly different compared to control ($P>0.05$; figure 5.1.2). Responses in R-R interval during control and 10 mmHg of neck pressure were of a slight decrease and a slight increase respectively.

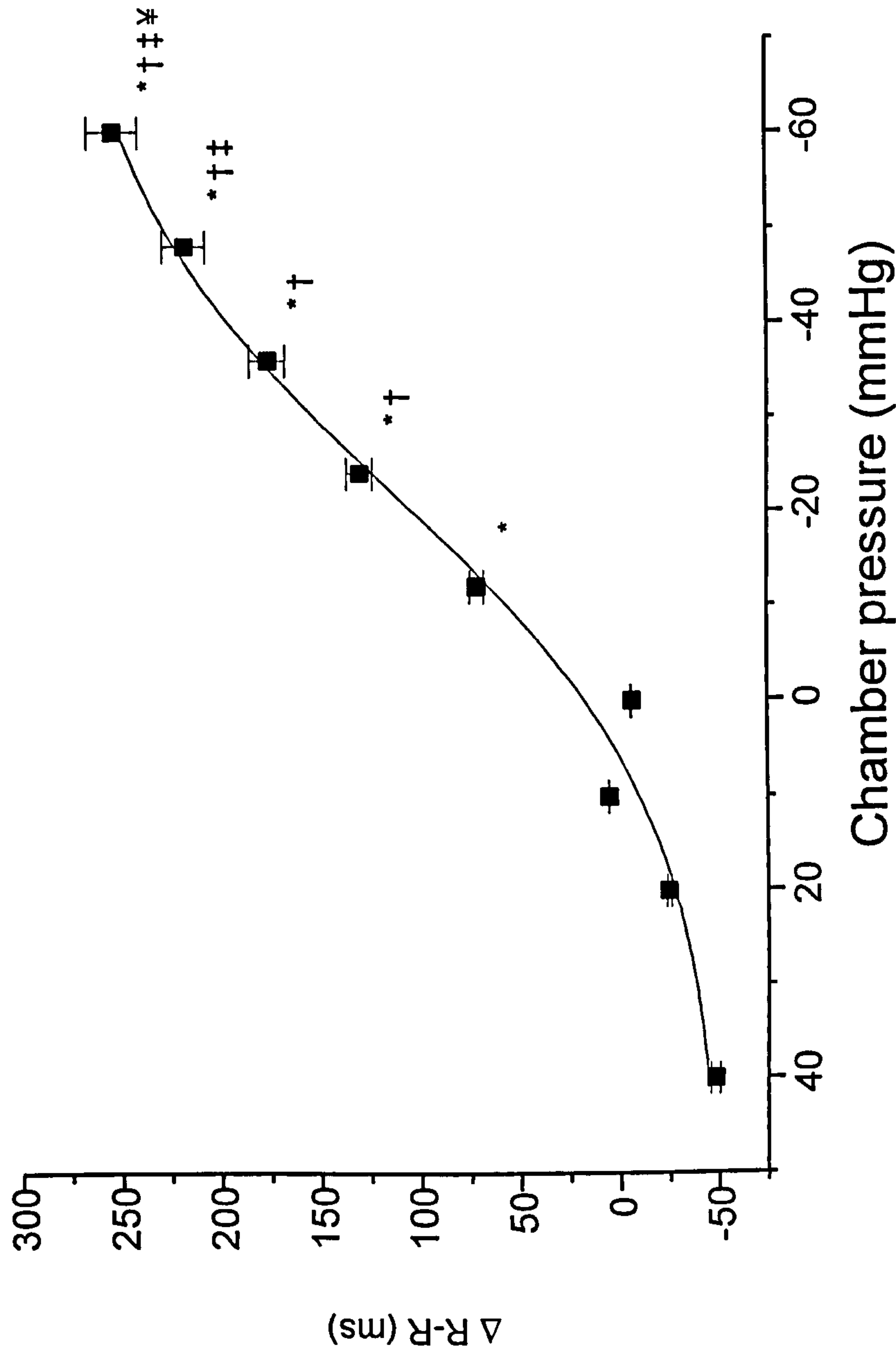


Figure 5.1.1. Responses in R-R interval to changes of internal neck chamber pressure from 40 to -60 mmHg for all subjects (χ^2 0.55). Data points are average \pm SE. * = Significant difference compared to control; † = significant vs -12mmHg; ‡ = significant difference vs -24mmHg; ¥ = significant difference vs -36mmHg (P <0.05).

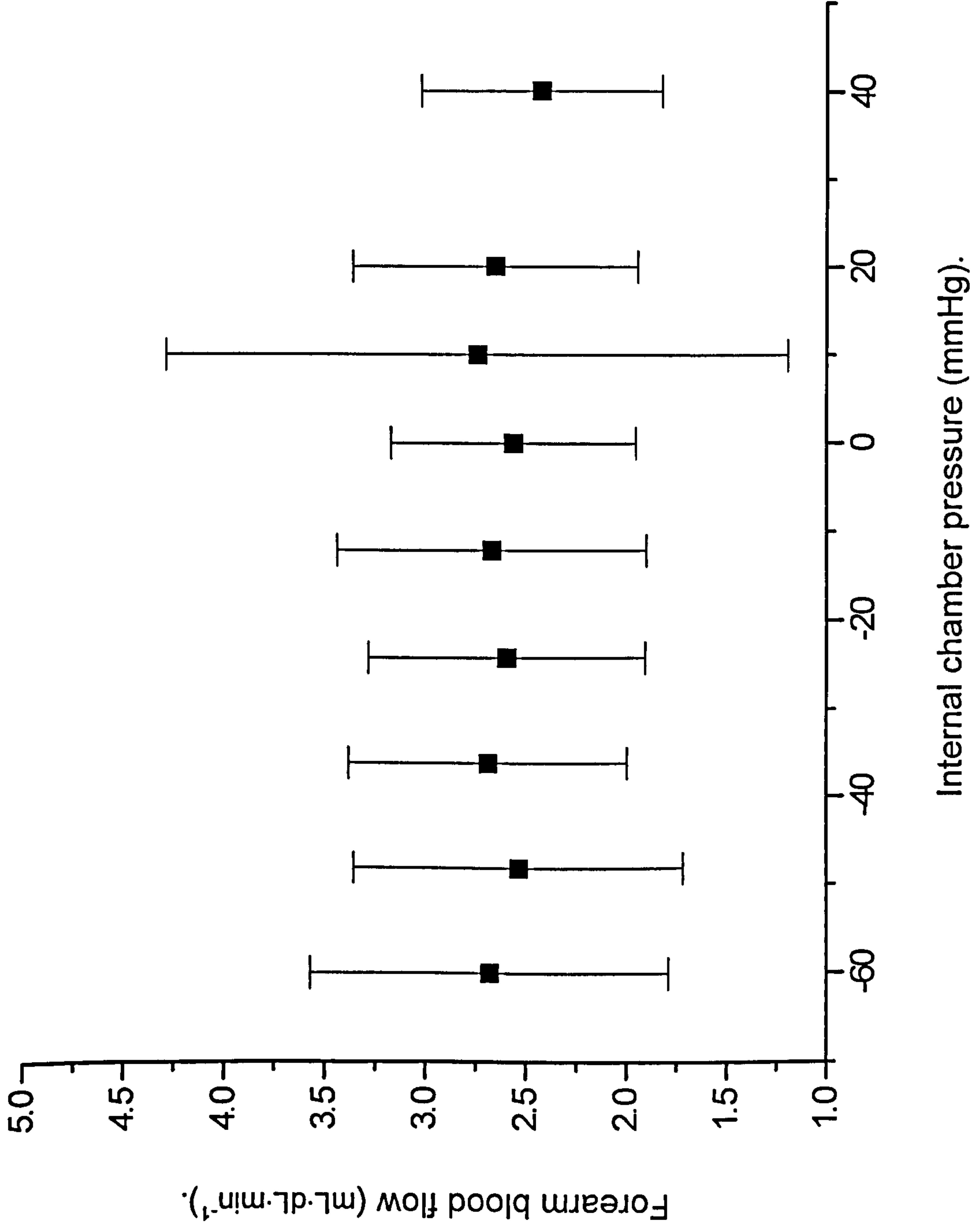


Figure 5.1.2. Forearm blood flow recorded during all stages of altered carotid sinus pressure. Values are group averages \pm SD. There were no significant differences between data points ($P>0.05$).

5.1.4 Discussion.

This study examined possible changes in forearm blood flow during a wide range of neck suction and pressure. There were no differences in FBF upon application of neck suction or during any of the neck pressure stages when compared to control ($P > 0.05$).

Quantification of carotid sinus baroreceptor stimulus.

There is confusion regarding the amount of negative or positive internal neck chamber pressure that is transmitted through the soft tissues of the neck to the carotid sinus baroreceptors. Estimates of this transfer range from 64 to 100% (Ludbrook et al., 1976; Eckberg, 1976). Since estimates of carotid sinus pressure are usually made by subtracting internal chamber pressure from systolic blood pressure, 100% transfer of chamber pressure to the carotid sinus baroreceptors would have to be assumed.

It has also been suggested that carotid sinus baroreceptors operate within a blood pressure range that is proportional to resting systolic blood pressure, and that these receptors reset to a higher range during hypertension (Eckberg and Sleight, 1992). Therefore, it is possible that carotid sinus baroreceptors respond to the magnitude of distending pressure change transmitted to this receptor region, rather than to a specific carotid sinus pressure. For these reasons internal chamber pressure was used to describe the stimulus presented to the carotid sinus baroreceptors in this study, since reporting estimates of carotid sinus pressure may be misleading.

Response in R-R interval to altered neck chamber pressure (carotid-cardiac baroreflex).

Previous studies that have used neck suction and pressure to stimulate and unload the carotid sinus baroreceptors, have reported a sigmoidal relationship between $\Delta R-R$ and estimated carotid sinus pressure (Eckberg, 1980; Pawelczyk et al, 1989; Convertino et al., 1990; Thompson et al., 1990; Lightfoot et al., 1994). In the present study, the relationship between $\Delta R-R$ and internal chamber pressure also fitted a sigmoidal function (figure 5.1.1). Therefore, it was assumed that the stimuli presented to subjects' carotid sinus, appeared to activate the baroreceptors in this region as demonstrated in previous work.

FBF responses to neck suction and neck pressure.

During all stages of carotid sinus baroreceptor stimulation or unloading FBF did not change significantly from control values. This finding supports previous studies that have reported non-significant, changes in FBF using either neck suction or electrical stimulation of the carotid sinus nerves (Epstein et al, 1969; Abboud et al, 1979; Ebert, 1982). However, the results of this study are in contrast to the work of Duprez et al (1987) who reported that blood flow in the finger reduced significantly with neck suction. Further, Ernsting and Parry (1957) noted a reduction in FBF with reduced pressure around the neck, but did not report the magnitude of this change.

Epstein et al (1969) and Ebert (1983) reported a reduction in forearm vascular resistance upon stimulation of carotid sinus baroreceptors, using an index calculated by dividing FBF into mean arterial pressure (MAP). However, both investigations found only minor changes in FBF, but much larger and significant reductions in

cardiac output. These changes in cardiac output are likely to be largely responsible for the noted changes in MAP. Therefore, it is possible that the reported reductions in forearm vascular resistance were attributable to changes in MAP and not reductions in forearm vascular tone.

Increases in forearm blood flow have been demonstrated during neck suction (Lindblad et al, 1982). However, these authors did not report values for changes in FBF, choosing instead to discuss forearm vascular resistance (FVR). Therefore, from their figure 3, it is difficult to assess the extent of the changes in FBF. In addition, electrical stimulation of the carotid sinus nerve has also elicited increases in FBF (Carlsten et al, 1958), but again the values for FBF were not reported.

Application of neck pressure did not produce any significant changes in FBF ($P>0.05$). Further, during neck pressures of up to 40mmHg, the change in carotid sinus distending pressure did not appear to be sufficient to elicit a significant change in R-R interval (and perhaps MAP). However, perhaps this is to be expected particularly when considering the carotid sinus baroreceptor-operating curve (Eckberg, 1980). It is also possible that the transfer of pressure across the neck tissue was inadequate.

The magnitude of Δ R-R during neck pressure at 40mmHg, though not significant, were almost identical to those reported by Sprenkle et al (1986), and therefore it appears that the neck pressure chamber used in this study was operating as originally intended. However, others (Victor and Mark, 1985; Vukasovic et al, 1990) have reported much larger and significant changes in heart rate or pulse interval

during neck pressure. This discrepancy may be due the use of neck chambers that were different in design (Victor and Mark, 1985) or a modified version of that described by Sprenkle et al (1986) (Vukasovic et al, 1990). Therefore, there may have been differences in the amount of chamber pressure transmitted to the carotid sinus baroreceptors.

5.1.5 Conclusion.

In this study neck suction of up to -60mmHg or neck pressure of up to 40mmHg did not provoke any significant changes in FBF. The response in R-R interval usually seen during this stimulus (Eckberg, 1980) was repeated in this study. Therefore it would appear as though the present stimuli altered carotid sinus distending pressure as intended. Hence from these data, carotid sinus baroreceptors, when stimulated or unloaded, do not appear to alter FBF as part of a reflex response to either neck suction or pressure.

5.2 The effect of hypercapnic acidosis on human CV responses and tolerance to orthostatic stress.

5.2.1 Introduction.

As discussed in chapter 1, selected mechanisms for human cardiovascular control of blood pressure and blood distribution during orthostatic stress have received considerable attention in recent years (Blomqvist and Stone, 1983; Raven et al, 1993). In particular responses of heart rate, cardiac output (Sather et al, 1986) and lower limb venous compliance (Coles et al, 1956; Wolthius, et al, 1975) to orthostatic stress have been studied as the principal mechanisms regulating cardiovascular responses to orthostatic stress.

In the preceding section in this chapter (5.1) it was demonstrated that forearm blood flow was not altered during stimulation or unloading of the carotid sinus baroreceptors. Therefore, these receptors may not play an important role in the peripheral vascular responses to orthostatic stress. However, the role of chemoreceptor control of peripheral blood flow has received little attention when investigating mechanisms associated with tolerance to orthostatic stress. Therefore, it remains possible that chemoreceptor reflexes may play an important role in human cardiovascular control during orthostatic stress, since it has been reported by Somers et al (1991) that a possible interaction exists between baroreflexes and chemoreflexes in the control of sympathetic nerve activity, which has been shown to

be a principal regulator of peripheral blood flow during orthostatic stress (Johnson et al, 1974).

There is confusion regarding the effect of stimulated chemoreceptors on the peripheral vasculature, indicated by changes in either forearm blood flow, total peripheral resistance or muscle sympathetic nerve activity (MSNA), which are important cardiovascular responses to orthostatic stress (Rowell, 1993). During hypercapnic acidosis, blood flow in the intact forearm has been shown to increase (Kontos et al, 1968a) or not change (Kontos et al, 1968b). Furthermore, total peripheral resistance during hypercapnic acidosis has been shown to increase (Daly et al, 1965), decrease (Rothe et al, 1990) or not change (Daugherty et al, 1967; Walker et al, 1990). However, only two of these investigations used human subjects (Kontos et al, 1968a; Kontos et al, 1968b), with the remainder being conducted using dogs (Daly et al, 1965; Daugherty et al, 1967; Rothe et al, 1990).

further studies, which induced hypercapnia, reported increases (Somers et al, 1989) and decreases (Shoemaker et al, 2001) in MSNA in human subjects. Therefore, there appears to be limited and equivocal data regarding the effect of hypercapnic acidosis on human peripheral vascular control during disturbances in blood distribution (orthostatic stress), and no published information regarding changes in calf circumference under hypercapnic conditions during orthostatic stress. Changes in forearm blood flow (Gilbert and Stevens, 1966; Johnson et al, 1974) and calf circumference (Coles et al, 1956; Wolthius et al, 1975) provide important information regarding the control of peripheral blood flow during orthostatic stress. However, the

role of the chemoreflexes in the regulation of peripheral blood flow during supine rest or orthostatic stress requires further investigation.

In this study forearm blood flow (FBF), calf circumference (CC) and cardiac responses were investigated while subjects were breathing room air in one condition and air containing 5% CO₂ in the other condition. The investigations were made during supine rest, followed by application of orthostatic stress (lower body negative pressure; LBNP) until the onset of presyncopal signs and symptoms (orthostatic tolerance; described in detail in chapter 2, section 2.5).

5.2.2 Methods.

Subjects.

Nine subjects [5 males and 4 females; average \pm SD, age 21.9 ± 0.9 years, height 172.4 ± 9.7 cm, mass 70.3 ± 7.1 Kg] volunteered to participate in this study following screening using a pre-participation medical questionnaire. Subjects gave written informed consent to participate in the study after receiving a verbal and written description of all experimental procedures, which were granted ethical approval by the De Montfort University Ethics Committee. All subjects reported to the laboratory at least four hours post-prandial and had avoided vigorous exercise for at least 24 hours. During all tests laboratory temperature and humidity were maintained ($23 \pm 0.8^\circ\text{C}$, $32 \pm 5\%$ respectively) and ambient barometric pressure was recorded from a mercury barometer for calculation of end tidal P_{CO_2} (see below).

Hypercapnia.

Throughout both normocapnic and hypercapnic conditions minute ventilation (V_E) and end tidal CO_2 (ET_{CO_2} ; expressed as a percentage of total end tidal expiratory gas) were measured using a Mass Spectrometer breath-by-breath gas analyser (Pulmolab EX 670, Morgan Medical Ltd, UK). This device is described in detail in chapter 2, section 2.19. ET_{CO_2} values were then used to calculate end tidal P_{CO_2} (P_{ETCO_2} ; % gas concentration/100 x ambient barometric pressure).

Hypercapnic acidosis was induced when subjects inspired 5% carbon dioxide (CO_2) in air (BOC gases, UK) from Douglas Bags through a low resistance valve. The low resistance valve was connected proximal to the volume turbine, capillary sampling

tube, and mouthpiece of the mass spectrometer (Pulmolab EX 670, Morgan Medical Ltd, UK), which was calibrated using known gases and volume syringe (see 2.19). Breathing 5% CO₂ in air has been shown previously to induce hypercapnic acidosis (Branco, 1998) and according to White et al (1952) this concentration of CO₂ increases P_{CO2} in alveolar air to approximately 47mmHg. Arterial P_{CO2} (P_{aCO2}) of at least 46mmHg is considered to indicate acidotic conditions (Tortora and Grabowski, 1996).

To assess estimates of P_{aCO2}, calculated from end tidal P_{CO2} (P_{ETCO2}; Fletcher and Boris-Möller, 2000), 2 males subjects (age 34.0 ± 2.8 years, height 172.8 ± 17.9 cm; mass 71.9 ± 14.2 Kg) were recruited and inspired 5% CO₂ from a single Douglas bag in the supine position for approximately 15 minutes, whilst ET_{CO2} (%) was recorded. Mean calculated P_{ETCO2} was 51.1 ± 3.9 mmHg and mean estimated P_{aCO2} was 56.5 ± 4.2 mmHg, thus indicating that these subjects were hypercapnic. Therefore, it was assumed that 5% CO₂ in air was a sufficiently high CO₂ concentration to induce hypercapnic acidosis in the subjects participating in this study.

When under the control condition (i.e. breathing room air), subjects were instrumented with the same volume turbine, capillary sampling tube and mouthpiece (as above), but breathing room air. To avoid an order effect in this study, a cross over study design was employed whereby four subjects were tested under control conditions first and five subjects were tested under hypercapnic conditions first.

Forearm blood flow and calf circumference.

Forearm blood flow (FBF) was determined using forearm occlusion plethysmography. Briefly, a mercury-in-silastic strain gauge was placed on the right forearm, distal to the lateral humeral condyle at the point of maximum forearm circumference. A small pneumatic cuff placed around the right wrist of the subject was inflated to a suprasystolic pressure to exclude circulation to the hand in the measurement of FBF. A second pneumatic cuff, placed around the upper right arm, which was inflated abruptly to 50 mmHg, thus occluding venous drainage from the arm. Three slopes, each during 8 seconds of venous occlusion, from the strain gauge were recorded during every third minute during tests. FBF was calculated as $\text{mL} \cdot \text{dL} \cdot \text{min}^{-1}$. FBF data are reported as percentage changes relative to pre-test levels. A detailed description of the equipment and procedures used for measures of FBF is given in chapter 2, section 2.16.

Calf circumference was calculated from changes in the signal output from a second mercury-in-silastic strain gauge placed around the right calf at the point of maximum circumference. The right calf was slightly elevated to avoid contact between the strain gauge and the foam padding inside the LBNP chamber. Changes in calf circumference were calculated (as described in chapter 2, section 2.17) and reported as percentage changes relative to pre-test calf circumference. Changes in calf circumference were used to indicate differences in the degree of pooled blood in the calf, in particular during orthostatic stress.

Cardiac output, stroke volume, heart rate, and electromyography.

Estimates of cardiac output (\dot{Q}) and stroke volume (SV) were made using measures of changes in thoracic impedance (Rheocardiometer, Rheo-Graphic PTE, Singapore) recorded from surface electrodes (see 2.20). The average percentage change in \dot{Q} and SV at every third minute during each test are reported in this chapter. A three-lead electrocardiograph was recorded from surface electrodes using an analogue to digital converter (PowerLab, AD Instruments, UK), from which heart rate (HR) was calculated instantaneously using specialised computer software (Chart v4.04, AD Instruments, UK; see 2.6.1). EMG was also recorded from three surface electrodes placed on the right vastus medialis muscle of subjects during all tests (see 2.6.2). This signal was used as a visual indicator of any lower body movement, which subjects were instructed to avoid.

Orthostatic stress test.

Upon arrival at the laboratory subjects adopted the supine position with their lower extremity placed inside the LBNP chamber and were sealed in at the level of the iliac crests and were then instrumented. Resting data were then recorded for a fifteen minute period during which subjects were either breathing room air (RA) through the mass spectrometer volume turbine and mouthpiece or breathing 5% CO₂ from additionally attached Douglas bags via the low resistance valve. This 15 minute rest period was necessary to ensure that possible changes in any or all of the variables described above had stabilised before application of the orthostatic stress. Following this 15 minute period, internal LBNP chamber pressure was reduced by 20mmHg for three minutes, after which a further reduction was made in chamber pressure of

10mmHg every three minutes until the onset of pre-syncopal signs and symptoms (see 2.5).

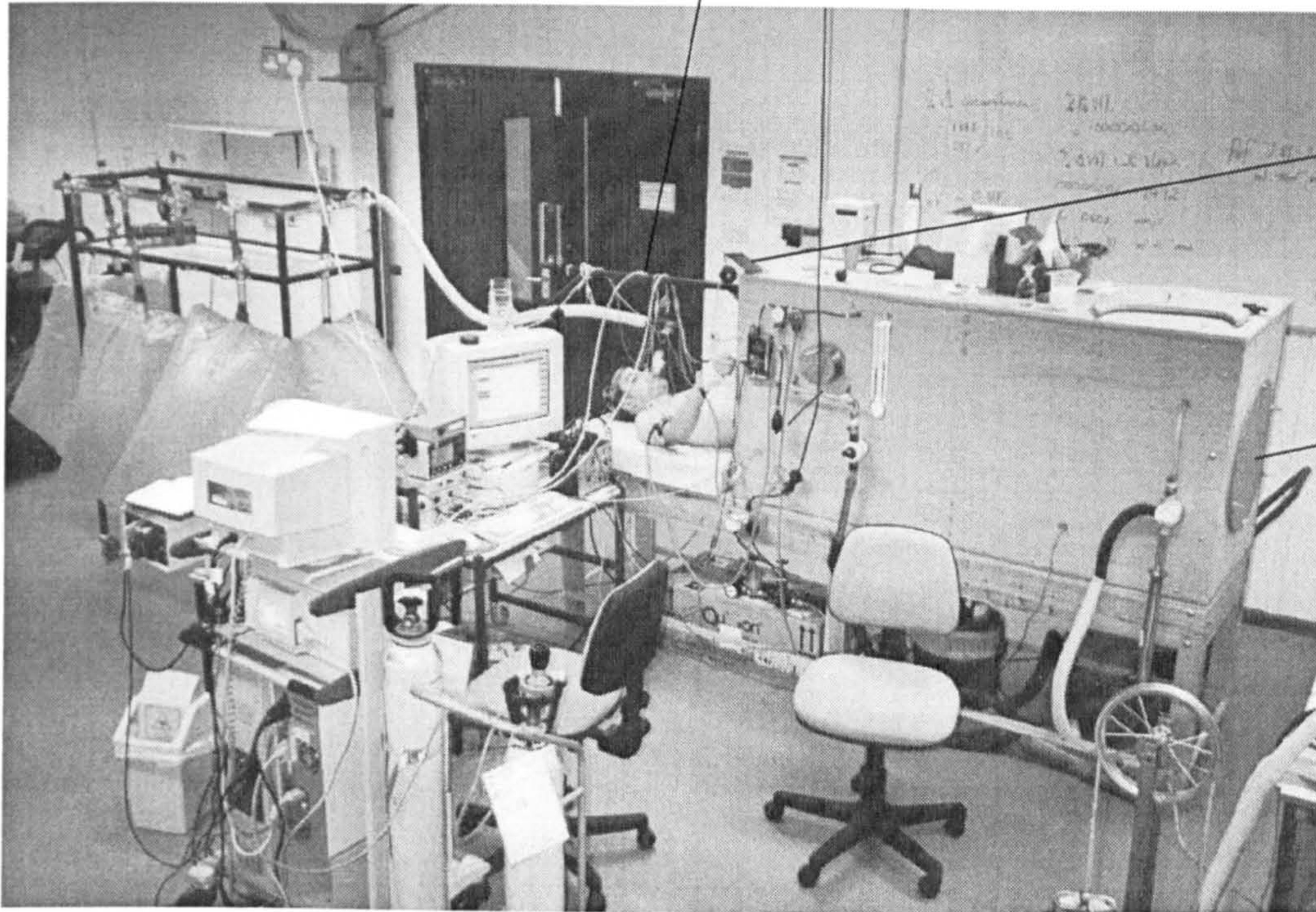
LBNP chamber modifications.

A number of small modifications were made to the LBNP chamber prior to investigations in this study. These modifications included a custom made support bar (760 x 25 x 25 mm) with bracket (120 X 25 x 2 mm) for attachment to the chamber, a specially fabricated pulley system that acted as an adjustable arm support so that the position of the subject's arm was identical between tests for measurement of FBF and a 390mm diameter hole cut in the end of the chamber to allow access to the subject's calf for instrumentation for measures of changes in calf circumference (figure 5.2.1). Figure 5.2.2 shows a subject sealed in the LBNP chamber, following instrumentation prior to the 15 minute control period and orthostatic stress test.

Statistical analysis.

Differences with in V_E , ET_{CO_2} , estimated Pa_{CO_2} , FBF, CC, \dot{Q} , SV and HR were assessed using a two-way repeated measures ANOVA with these variables categorised by time (i.e. each time point where data was recorded during both rest and orthostatic stress) and gas (i.e. breathing room air or 5% CO_2). Variables that showed significant differences either by time, gas or time x gas interaction ($P < 0.05$), Fisher's protected LSD post-hoc test was used to assess where the differences were. Differences between LTI, peak HR and time to peak HR during orthostatic stress when breathing either room air or 5% CO_2 were assessed using a paired student's *t*-test.

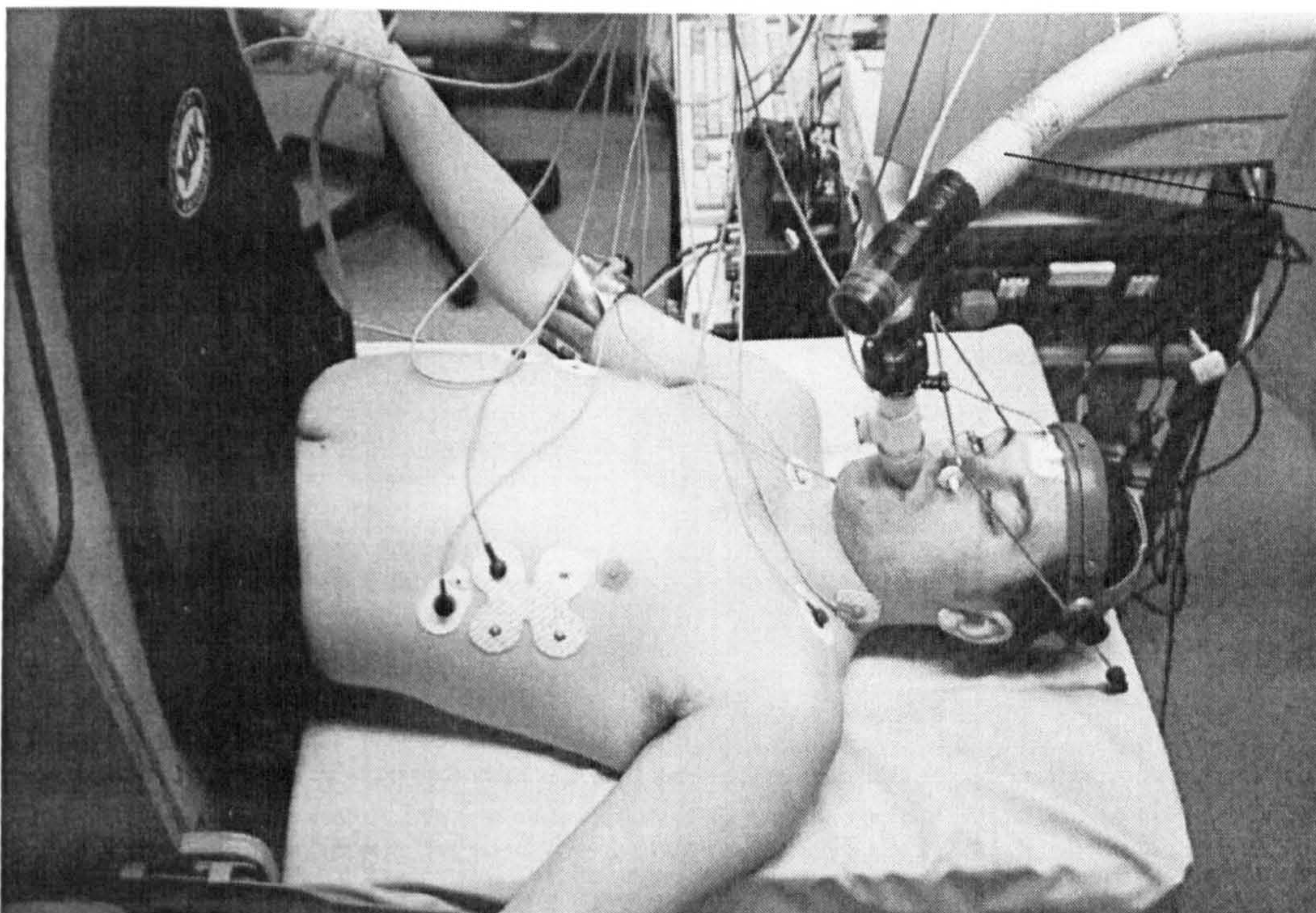
Support bar for tubing and cables



Adjustable
arm support

Access to
subject's calf

Figure 5.2.1 Showing modifications to the LBNP chamber made to conduct the present study, including; adjustable arm support for FBF measures, bar supporting tubing to douglas bag rack and cables, and the hole cut in the end of the chamber (covered in picture) to allow access to the subject's calf for instrumentation.



Tubing through
which 5% CO₂ in
air was
introduced

Figure 5.2.2 Showing subject sealed in chamber and instrumented for FBF, ECG, thoracic impedance and custom built system for introducing 5% CO₂ in air to the subject whilst recording V_E and ET_{CO_2} .

5.2.3 Results.

In this study orthostatic tolerance, cardiovascular and respiratory responses were assessed during normocapnia and hypercapnia. However, subjects' tolerance to orthostatic stress was varied and not all subjects were able to tolerate the same magnitude of orthostatic stress. Therefore, when time course changes in the above variables during rest and orthostatic stress are expressed below, values up to and including the commonly tolerated maximum negative pressure are reported. This was necessary to avoid reductions in the subject group size, if data recorded during higher levels of orthostatic stress were reported when not all subjects were able to tolerate these higher levels of stress. The limitations associated with this method of data analysis are accepted despite the comments regarding this method in chapter 3. Variables that are reported as single values for each test (e.g. peak heart rate, time to peak heart rate, SV and Q during orthostatic stress) were calculated from the LBNP data up to, but excluding, presyncopal values since these were often depressed.

Furthermore, responses at both rest and during orthostatic stress were assessed whilst breathing room air and 5% carbon dioxide in air. However, these assessments were made with five to seven days between tests. Therefore, in order to avoid day to day fluctuations in the variables assessed in this study interfering with the pattern of responses to the two conditions, relative change in FBF, CC, HR, SV and Q during tests are reported.

Cardiovascular and ventilatory responses to hypercapnia during supine rest.

There were no significant relative changes in heart rate (HR; 9.03 ± 21.59 vs 9.01 ± 21.91 vs 8.83 ± 21.56 vs 9.57 ± 21.02 [5% CO₂] and, 1.10 ± 3.32 vs 1.18 ± 3.93 vs 2.00 ± 5.50 vs 1.01 ± 4.25 [RA] %), stroke volume (SV; 1.97 ± 8.17 vs 2.48 ± 11.65 vs 1.90 ± 11.53 vs 2.06 ± 12.61 [CO₂] and vs, -0.47 ± 9.15 vs -2.29 ± 3.49 vs -4.34 ± 8.04 vs 1.04 ± 16.71 [RA] %) and therefore cardiac output (\dot{Q} ; 4.39 ± 9.94 vs 5.23 ± 9.85 vs 7.19 ± 9.29 vs 7.84 ± 13.13 [CO₂] and vs, 0.70 ± 7.89 vs 3.42 ± 3.26 vs 2.86 ± 5.15 vs 2.99 ± 18.13 [RA]%) during hypercapnia when compared to normocapnia or across time whilst in the supine position ($P>0.05$; figure 5.2.3). Further there were no changes in forearm blood flow (FBF; 4.16 ± 24.88 vs -0.36 ± 21.47 vs -2.34 ± 12.02 vs -9.17 ± 20.13 [CO₂] and vs, -2.60 ± 11.75 vs -3.95 ± 14.16 vs -10.08 ± 15.09 vs -3.91 ± 18.93 [RA]%) and calf circumference (CC; 0.01 ± 0.03 vs 0.06 ± 0.03 vs 0.09 ± 0.05 vs 0.09 ± 0.14 [CO₂] and vs, 0.05 ± 0.03 vs 0.1 ± 0.04 vs 0.15 ± 0.04 vs -0.18 ± 0.08 [RA]%) when breathing either 5% CO₂ or RA, or between these two conditions ($P>0.05$; figure 5.2.4).

Minute ventilation (V_E), end tidal CO₂ (ET_{CO2}) and estimated arterial P_{CO2} (Pa_{CO2}) were significantly elevated when breathing 5% CO₂ when compared to breathing room air (RA) during supine rest (table 5.2.1; $P<0.001$). Whilst breathing 5% CO₂ there was a significant increase in V_E during supine rest from minute 6 when compared to minute 3, which plateaued between the 12 and 15 minute time points ($P<0.05$, table 5.2.1)

Tolerance to orthostatic stress.

All subjects tolerated LBNP until the onset of pre-syncopal signs and symptoms and no tests were terminated as a result of discomfort experienced by the subject or equipment failure. The recorded EMG signal did not indicate any lower body movement by subjects during orthostatic stress. There was a significant increase in orthostatic tolerance when subjects were breathing 5% CO₂ in air when compared to breathing RA ($P<0.05$; table 5.2.2).

Cardiovascular and ventilatory responses to hypercapnia during orthostatic stress.

There was a significant relative increase in HR from -30 mmHg of LBNP (21.8 ± 18.0 vs 30.2 ± 17.3 vs 42.4 ± 17.7 % [5%CO₂] and 15.2 ± 6.7 vs 25.4 ± 8.0 vs 44.3 ± 12.8 % [RA]) and reductions in SV from -20mmHg of LBNP (-13.6 ± 15.5 vs -24.7 ± 21.2 vs -37.6 ± 16.8 vs -50.4 ± 10.7 % [CO₂] and -11.8 ± 9.2 vs -26.2 ± 12.1 vs -40.2 ± 9.2 vs -52.6 ± 9.2 % [RA]) and \dot{Q} from -30mmHg of LBNP during hypercapnia (-16.8 ± 19.6 vs -24.4 ± 15.8 vs -33.4 ± 12.2 %) and from -20mmHg of LBNP during normocapnia (-11.6 ± 8.9 vs -17.6 ± 11.0 vs -27.4 ± 9.6 vs -34.1 ± 11.3 %) when compared to the first period of data collection during supine rest ($P<0.05$; figure 5.2.3), but this change was not different between the two conditions ($P>0.05$; figure 5.2.3). Just prior to presyncope, peak HR was significantly higher during hypercapnia when compared to normocapnia ($P<0.05$; table 5.2.2). This higher peak HR was reflected in an increase in the time to peak HR, from the onset of orthostatic stress, during hypercapnia compared to normocapnia ($P<0.05$; table 5.2.2) in addition to a further 22.3% reduction in SV during hypercapnia (table 5.2.2).

There was a significant relative reduction in FBF (-34.3 ± 21.2 vs -40.1 ± 26.9 vs -44.0 ± 19.5 vs -48.4 ± 20.3 % [CO_2] and -38.6 ± 15.4 vs -44.6 ± 20.8 vs -55.4 ± 15.1 vs -57.2 ± 14.8 % [RA]; $P < 0.05$; figure 5.2.4) during all stages of orthostatic stress when compared to the first period of data collection during supine rest, but these responses were not different between gases ($P > 0.05$; figure 5.2.4). However, CC increased significantly from the second stage of orthostatic stress (-30 mmHg of LBNP) during hypercapnia (0.4 ± 0.3 vs 0.6 ± 0.3 vs 0.9 ± 0.3 %; $P < 0.05$; figure 5.2.4) and from -20 mmHg of LBNP during normocapnia (0.3 ± 0.2 vs 0.6 ± 0.3 vs 0.9 ± 0.3 vs 1.2 ± 0.3 %; $P < 0.05$; figure 5.2.4). This increase was significantly greater at -50 mmHg of LBNP when subjects were breathing RA compared to 5% CO_2 ($P < 0.05$; figure 5.2.4).

During orthostatic stress, P_{ETCO_2} and estimated P_{aCO_2} did not show any further changes when compared to values recorded during supine rest ($P > 0.05$; table 5.2.1). However, V_E increased above hypercapnic resting levels during orthostatic stress once the -30 mmHg level of LBNP had been reached ($P < 0.001$; table 5.2.1). V_E when breathing RA did not change at any stage of the experiment ($P > 0.05$; table 5.2.1).

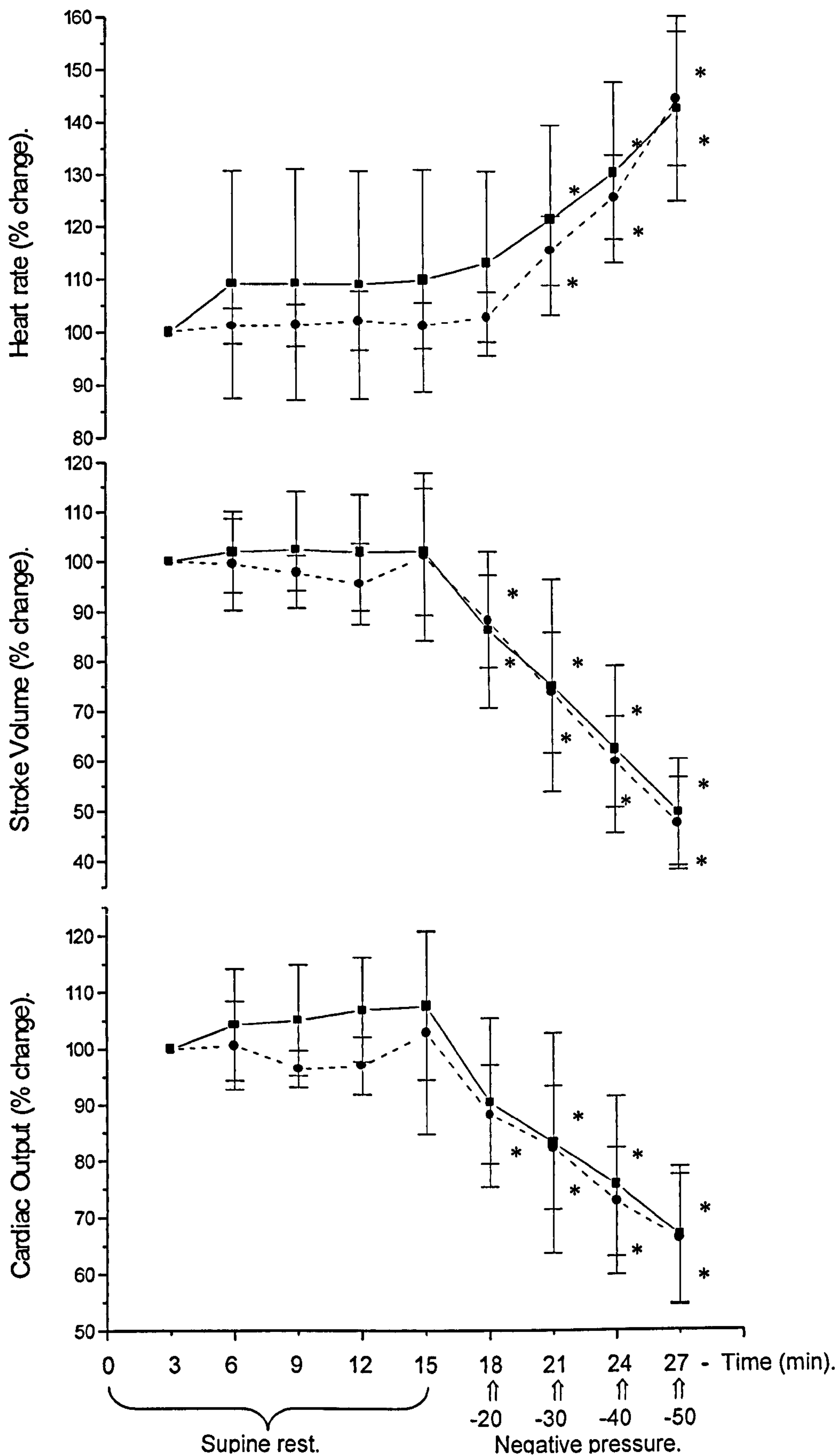


Figure 5.2.3. Heart rate (HR, stroke volume (SV) and cardiac output (Q) during supine rest and orthostatic stress up to -50mmHg of LBNP (average \pm SD) during hypercapnia (■) and normocapnia (●). * = significant time course change from minute 3.

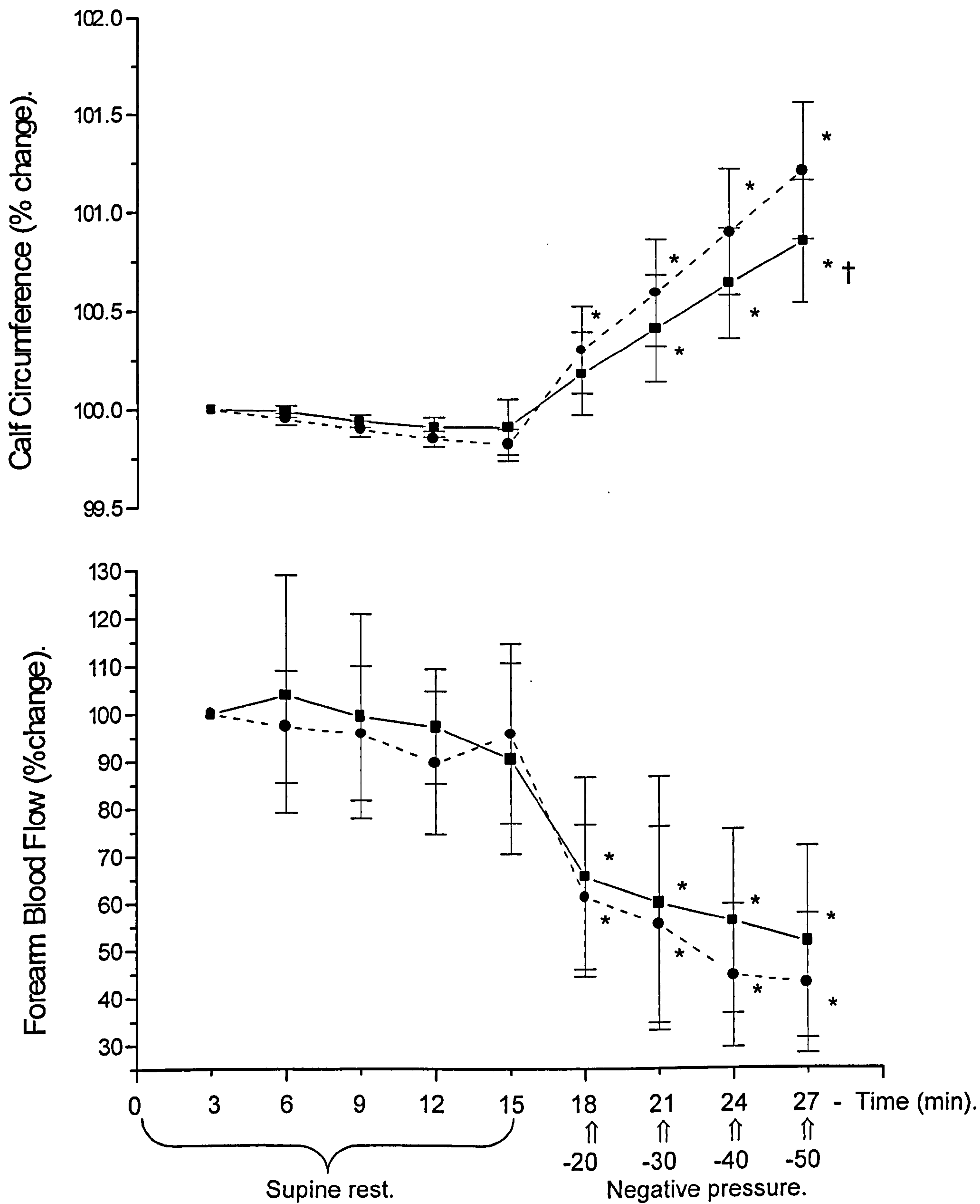


Figure 5.2.4. Forearm blood flow (FBF) and calf circumference (CC) during supine rest and orthostatic stress up to -50mmHg of LBNP (mean \pm SD) during hypercapnia (■) and normocapnia (●). * = significant time course change from minute 3. † = significant difference from normocapnia.

Table 5.2.1. Differences in respiratory variables measured when breathing room air or 5% carbon dioxide in air during rest and orthostatic stress of up to -50mmHg of LBNP. Values (average \pm SD) for minute ventilation (V_E), end tidal CO_2 (ETCO₂) and estimated arterial PCO₂ (Paco₂). * = significantly different when breathing 5% CO₂ compared to breathing RA (P < 0.001). † = significantly different from the measure at 3 minutes (P<0.05).

Time (min or mmHg)			Breathing room air (RA)			Breathing 5% carbon dioxide (5% CO ₂)		
			V_E (L·min ⁻¹)	P _{ETCO₂} (mmHg)	Paco ₂ (mmHg)	V_E (L·min ⁻¹)	P _{ETCO₂} (mmHg)	Paco ₂ (mmHg)
3			4.63 \pm 1.63	41.40 \pm 3.87	45.48 \pm 4.22	12.29 \pm 3.53*	51.97 \pm 1.96*	57.01 \pm 2.13*
6			4.90 \pm 1.57	40.89 \pm 5.61	44.93 \pm 6.12	14.38 \pm 2.59*	52.42 \pm 1.70*	57.50 \pm 1.85*
9			4.66 \pm 1.88	42.79 \pm 2.06	47.00 \pm 2.24	15.74 \pm 2.18*†	52.41 \pm 2.14*	57.49 \pm 2.33*
12			5.17 \pm 1.66	41.85 \pm 2.46	45.97 \pm 2.68	16.71 \pm 2.11*†	52.30 \pm 2.10*	57.37 \pm 2.29*
15			5.19 \pm 1.62	42.08 \pm 3.20	46.23 \pm 3.49	16.70 \pm 3.54*†	51.57 \pm 3.99*	56.57 \pm 4.35*
18 (-20)			4.21 \pm 1.31	40.71 \pm 4.92	44.74 \pm 5.36	17.65 \pm 3.39*†	51.43 \pm 1.83*	56.42 \pm 1.99*
21 (-30)			4.76 \pm 0.83	40.03 \pm 2.09	44.00 \pm 2.27	17.62 \pm 2.19*†	50.90 \pm 2.00*	55.84 \pm 2.18*
24 (-40)			4.80 \pm 1.35	40.34 \pm 2.04	44.33 \pm 2.22	18.07 \pm 1.83*†	50.23 \pm 2.39*	55.11 \pm 2.60*
27 (-50)			4.99 \pm 1.14	40.60 \pm 2.35	44.61 \pm 2.56	19.99 \pm 3.05*†	49.52 \pm 2.47*	54.34 \pm 2.70*

Table 5.2.2. Orthostatic tolerance, peak HR during orthostatic stress and cardiac dynamics immediately prior to presyncope. Values (average \pm SD). * = significantly different when breathing 5% CO₂ compared to breathing RA (P < 0.05).

Breathing RA.		Breathing 5% CO ₂ .
LTI (Δ mmHg \cdot min).	191.9 \pm 20.4	210.3 \pm 20.9*
Peak HR (b \cdot min ⁻¹).	123.9 \pm 23.7	131.9 \pm 24.9*
Time to peak HR (min).	16.1 \pm 2.1	18.1 \pm 1.8*
SV (mL).	37.3 \pm 2.1	29.0 \pm 6.7
Q (L \cdot min).	3.9 \pm 0.9	3.7 \pm 0.7

5.2.4 Discussion.

In this study the inspiration of air containing 5% carbon dioxide (CO_2) was accompanied by significant increases in minute ventilation (V_E), end tidal CO_2 (ET_{CO_2}) and estimated arterial P_{CO_2} (Pa_{CO_2}) indicating that subjects were in a hypercapnic state. Under hypercapnic conditions there was an increase in orthostatic tolerance, peak HR and time to peak HR during orthostatic stress, when compared to normocapnia. During orthostatic stress of up to -50mmHg of LBNP, there were increases in minute ventilation, heart rate and calf circumference (CC). The increase in calf circumference was attenuated at -50mmHg of LBNP during hypercapnia compared to normocapnia. Furthermore, there were reductions in forearm blood flow (FBF), stroke volume (SV) and cardiac output (\dot{Q}) during orthostatic stress that were not different between the two conditions.

Cardiovascular and ventilatory responses to hypercapnia during supine rest.

Hypercapnia has been reported previously to result in increases in heart rate (White et al, 1952; Kontos et al, 1968a) and \dot{Q} (Rothe et al, 1989; Rothe et al, 1990) and reduced peripheral vascular resistance (Kontos et al, 1968b; Neubauer et al, 1978; Rothe et al, 1990). However, other reports have shown these changes in peripheral vascular resistance to be equivocal (Daugherty et al, 1967; Kontos et al, 1968a; Walker and Brizzee, 1990).

During the rest period in this study there were no significant changes in FBF, CC, HR, SV or \dot{Q} under normocapnic or hypercapnic conditions. There is limited and inconsistent data available with regard to human cardiovascular responses to

hypercapnia. In previous studies of humans, hypercapnic acidosis has been induced by either breathing 7% CO₂ in air (Kontos et al, 1968a) or intra-arterial infusion of monosodium-disodium phosphate buffer solutions (Kontos et al, 1968b). The difference in these methods may explain the lack of consistency in peripheral vascular resistance (FBF) responses to hypercapnia. This suggests that breathing 5% CO₂ (this study) or 7% CO₂ (Kontos et al, 1968a) in air does not induce sufficient acidosis to effect a measurable change in FBF in humans, although breathing 5% CO₂ in air has been shown to increase blood flow in rat muscle (Neubauer et al, 1978). However, the magnitude of CO₂ concentration in air may not provide an explanation for the lack of changes in FBF during 5% CO₂ breathing, since Daugherty et al (1967) has reported little effect of breathing 20% CO₂ in air on the calibre of limb vessels of the dog.

FBF has been shown to increase during hypercapnia (CO₂ breathing) following β_2 – adrenergic receptor blockade suggesting that CO₂ has an effect on local regulation of blood flow (Kontos et al, 1967). Furthermore, sympathetic nerve activity (SNA) increases during hypercapnia (Somers et al, 1991; Somers et al, 1989), which may oppose the previously reported vasodilatory effect of CO₂. Therefore, the data of the present study offers support for previous suggestions of the competing effects of local vasodilatation and an increase in SNA which appear to cancel out each other and result in no change in FBF (Kontos et al, 1967).

From figure 5.2.1 it can be seen that although there was not a significant increase in HR during hypercapnia, HR appears to rise sharply from minutes 3 to 6. Previous studies that have reported increases in HR under hypercapnic conditions used a slightly higher concentration CO₂ (6% CO₂ in air, White et al, 1952; 7% CO₂ in air,

Kontos et al, 1968a). Therefore it is possible that breathing 5% CO₂ in air was not a sufficiently high concentration to induce significant changes in HR at rest.

Increases in \dot{Q} have been reported previously during hypercapnia in dogs (Rothe et al, 1989; Rothe et al, 1990). However, there is limited data regarding \dot{Q} and SV responses to hypercapnia in normal humans. Shoemaker et al (2001) reported no change in \dot{Q} or SV during hypercapnia with a P_{ETCO_2} of 45 mmHg ($P_{aCO_2} \approx 49$ mmHg), which was a slightly lower estimated P_{aCO_2} during hypercapnia in the present study ($P_{aCO_2} \approx 56$ mmHg). However, significant increases in \dot{Q} have been reported previously, but during a much higher P_{aCO_2} (74mmHg; Djurberg et al, 1998). Therefore, this suggests that large increases in P_{aCO_2} are required to elicit changes in \dot{Q} and perhaps SV.

The changes in ET_{CO_2} , estimated P_{aCO_2} , and subsequent influence on V_E during hypercapnia were as expected based on previous reports (De Burgh Daly, 1986; White, 1954; Heller et al, 1929). Since these were the only significant changes during hypercapnia immediately prior to application of orthostatic stress they may provide a partial explanation for the differences between the two conditions during orthostatic stress.

Cardiovascular and ventilatory responses to hypercapnia during orthostatic stress.

There was a significant increase in orthostatic tolerance during hypercapnia compared to normocapnia. This improved tolerance to orthostatic stress was not accompanied by differing responses in HR, SV or \dot{Q} up to -50mmHg of LBNP.

These data are in agreement of the data of Shoemaker et al (2001) which also demonstrated that hypercapnia does not affect the responses of the heart to non-presyncopal orthostatic stress. However, peak HR and the time to peak HR just prior to presyncopal symptoms in this study were significantly greater during hypercapnia. In chapter 4, time to peak HR was associated with increases in orthostatic tolerance previously following isometric exercise training of the legs and it was suggested that this may have been associated with an improved maintenance of venous return to the heart during orthostatic stress.

Indeed, at -50mmHg of LBNP the increase in calf circumference (CC) was significantly less during hypercapnia when compared to normocapnia. This suggests that venous return was perhaps better maintained under hypercapnic conditions during orthostatic stress, perhaps induced by increased SNA. However, the data of Shoemaker et al (2001) suggests a slightly diminished MSNA response to orthostatic stress. Moreover, this difference in CC dynamics between the two conditions in this study was not reflected by larger SVs or lower HRs at -50mmHg of LBNP. Furthermore, the relative changes in CC at the time of the last measurement prior to presyncope were not different between the two conditions. The absence of an enhanced response in MSNA to orthostatic stress under hypercapnic conditions was perhaps reflected by the similar response in FBF during orthostatic stress. Therefore, it appears that the dynamic changes in these two factors were not related to each other and perhaps other responses to hypercapnia were responsible.

The respiratory pump is known to be an important factor in maintaining venous return during orthostatic stress (Rowell, 1993). In this study, during hypercapnia, V_E was

significantly elevated when compared to normocapnia. Furthermore, V_E continued to increase during orthostatic stress and may have played an increasingly important role in venous return as the orthostatic stress became more severe. However, it seems likely that this would have been accompanied by an attenuated reduction in SV and \dot{Q} immediately prior to presyncope, which did not occur in this study. Indeed, the reduction in SV under hypercapnic conditions during orthostatic stress was 22.3% greater than the SV reduction during normocapnia. Furthermore, De Burgh Daly (1986) has suggested that deep and rapid breathing may induce excessive emptying of the vena cavae, thus reducing central venous pressure. With an accompanying translocation of blood into the lower limbs during orthostatic stress, also known to reduce central venous pressure (Johnson et al, 1974), the increase in V_E observed in this study would likely be associated with an accelerated loss of central venous pressure and early pre-syncope. Since the effect of hypercapnia was to increase tolerance to orthostatic stress, other factors are likely to be responsible for a delay in the onset of pre-syncopal signs and symptoms resulting in greater reductions in SV and increases in HR.

The effect of P_{aCO_2} on cerebral blood flow velocity and therefore cerebral blood flow (Serrador et al, 2000) is well known and a 40-50% increase in cerebral blood flow (CBF) has been suggested when breathing 5% CO_2 in air (Rowell, 1986). However, during orthostatic stress, CBF has been shown to decrease (Bondar et al, 1991; Grubb et al, 1991; Zhang et al, 1997; Serrador, 2000) and authors have suggested that this reduction in CBF, below a point at which cerebral arterial pressure can no longer be maintained, initiates the onset of pre-syncope (Glaister and Miller, 1990; Grubb et al, 1991). This reduction in CBF during orthostatic stress has been

associated with reductions in P_{aCO_2} at pre-syncope induced by hyperventilation (Morgan et al, 1997; Imms, 2000). Therefore, it is possible that under hypercapnic conditions with elevated P_{aCO_2} , thus increasing CBF, the established reduction in CBF was delayed resulting in a later onset of pre-syncope. Whilst this suggestion is speculative, it is at least supported by the further reduction in SV during orthostatic stress and the greater increase in HR, which suggests a greater magnitude of central hypovolaemia.

The occurrence of decreased P_{aCO_2} , induced by hyperventilation, at pre-syncope (Morgan et al, 1997; Imms, 2000) could be stimulated by a given degree of central hypovolaemia during orthostatic stress. The increase in V_E during early orthostatic stress under hypercapnic conditions observed in this study, may suggest a change in the sensitivity of the chemoreflex. It is possible that the mechanism for hyperventilation at pre-syncope, perhaps a baroreceptor/chemoreceptor interaction (Heistad et al, 1975), was more sensitive upon chemoreceptor stimulation to the reduction of central blood volume. Therefore, hyperventilation, in an attempt to reduce P_{aCO_2} , has occurred during early orthostatic stress and lower levels of central hypovolaemia in this study. However, since during the hypercapnic condition inspired air contained 5% CO_2 , it could be suggested that the increasing V_E during orthostatic stress was ineffective in reducing P_{aCO_2} and thus the onset of presyncope could have been initiated by alternative mechanisms that may be redundant under normal conditions.

5.2.5 Conclusion.

In this study breathing 5% CO₂ in air induced hypercapnic acidosis in subjects. Hypercapnia resulted in increases in estimated Pa_{CO2} leading to increased ventilatory responses at rest and during orthostatic stress. Hypercapnia did not induce any changes in the haemodynamic factors associated with tolerance to orthostatic stress during supine rest. However, there was an increase in orthostatic tolerance, which may have been related to increases in CBF, thus delaying the onset of pre-syncopal signs and symptoms when compared to the normocapnic condition.

Chapter 6

General Discussion.

6.0 General discussion.

In this thesis, three main aspects of human cardiovascular (CV) control during simulated or related orthostatic stresses have been studied. The three aspects of human CV control studied were repeated exposure to orthostatic stress, manipulation of resting blood pressure and receptor stimulation performed on human subjects. In the proceeding discussion, emphasis will be placed on tolerance to orthostatic stress, in addition to factors affecting blood pressure homeostasis and disturbances in total or central blood volume as the three principal elements affecting the maintenance of consciousness during orthostatic stress.

As discussed in chapter 1, tolerance to orthostatic stress (orthostatic tolerance) refers to the maximum tolerated orthostatic stress immediately prior to syncope. At syncope, blood pressure homeostasis is no longer possible and thus consciousness is lost. In recent years it has become common to define orthostatic tolerance as the occurrence or onset of pre-syncopal signs and symptoms (Murray et al, 1968; see chapter 2). The most common pre-syncopal signs and symptoms presented by subjects reported in this thesis included precipitous reductions in heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP), which also appear to be common in previous studies (Lightfoot et al, 1989; Lightfoot et al, 1991). In addition it has been reported previously that there is an abrupt increase in forearm blood flow (FBF) at pre-syncope (Dietz et al, 1997), although FBF was not measured specifically at pre-syncope in the present studies.

Under conditions of orthostatic stress (such as LBNP) there is a translocation of blood from the central circulation into the dependant vessels of the lower limbs (Wolthius et al, 1975), leading to reductions in SBP, increases in DBP resulting from peripheral vasoconstriction and increases in HR (Stevens, 1966; Lightfoot et al, 1991; Rowell, 1993). Although, vasoconstriction in the forearm does not appear to be controlled by the unloaded carotid sinus baroreceptors (see 5.1). In subsection 5.2, the demonstrated increase in calf circumference, the reductions in SBP in subsection 3.1, and the increases in HR in chapters 3-5, during orthostatic stress, provide evidence of the established lower limb blood pooling and reductions in central blood volume (Wolthuis et al, 1975) and pressure.

Prior to the onset of pre-syncope in these studies, there were established responses in heart rate (HR), stroke volume (SV), cardiac output (\dot{Q}), blood pressure (BP), forearm blood flow (FBF), and calf circumference (CC; see chapters 3, 4 and 5) during orthostatic stress that indicated a loss of central blood volume and pooling in the lower extremity. Under these conditions, responses in HR and of the vasculature of the forearm were manifestations of human CV control of BP homeostasis and avoidance of pre-syncope. Furthermore, there were established responses in R-R interval during neck suction and pressure, but no change in FBF was observed.

Pre-syncopal signs and symptoms (see chapter 2) are collectively referred to as the vasovagal reflex or response, and are the combination of peripheral vasodilatation and a slowing of HR, thus inducing rapid reductions in blood pressure, indicating impending syncope (van Lieshout et al, 1991). The frequency with which these signs

and symptoms occurred was demonstrated in subsection 3.1 when 81% of all orthostatic stress tests were terminated upon the occurrence of one or more of these events. In the subsequent studies, these pre-syncopal signs and symptoms did not appear to differ after repeated exposure to orthostatic stress, BP manipulation, or concomitant hypercapnia. This provides further evidence for the usefulness of these signs and symptoms for indicating *impending* syncope, particularly since there were no incidences of syncope in the present studies. The remainder of orthostatic stress tests were terminated after subject request due to light-headedness or severe nausea. The length of time and magnitude of negative pressure (which are used to calculate LTI, as described in chapter 3) tolerated by subjects appeared to vary according to manipulations and adaptations to the CV systems of subjects presented in this thesis.

6.1 The effect of exposure to orthostatic stress on orthostatic tolerance.

Orthostatic tolerance and sustained consciousness are known to be dependant on the control of the translocation of blood into the vessels of the dependant limbs (Amberson, 1943; Coles et al, 1956; Wolthius et al, 1975; Halliwill et al, 1998). Regulation of the subsequent disturbance in central blood volume essentially comprises of responses of the heart and peripheral vasculature as the human CV system attempts to maintain arterial BP and adequate cerebral perfusion (Zhang et al, 1997; Sung et al, 2000). However, there is little available data with regard to the effect of orthostatic stress itself on the human CV system and the influence that these effects may have on tolerance (Hilton et al, 1988; Lightfoot et al, 1989; Lightfoot et al, 1991). Furthermore, there appears to be a general acceptance that human CV

responses to orthostatic stress are reproducible despite some of the available data suggesting that orthostatic stress itself can induce CV adaptations that affect tolerance (Hilton et al, 1988; Lightfoot et al, 1989). In addition, the quantity of data concerning the reproducibility of human CV responses to orthostatic stress is not sufficient for investigators to be confident with regard to repeatability of these responses.

Chapter 3 detailed two studies that examined, first (3.1) the reproducibility of tolerance to orthostatic stress, and second (3.2), possible mechanisms that may explain the results of the first study (i.e. an increase in orthostatic tolerance). The conflicting results from these studies could have added confusion to the matter of reproducibility of orthostatic CV responses. The study of 3.1 potentially suggests an adaptive response to orthostatic stress despite allowing 3-5 days between tests. Conversely, when tests were separated by only 24 hours, no adaptation to orthostatic stress was observed. However, it would appear from the data in 3.1 and that of Lightfoot et al (1989), that tolerance in a small core of subjects increases following repeated exposure to orthostatic stress, typically occurring after the second test, but did not occur when tests were repeated daily (3.2). This suggests that the test-retest study designs of the subsequent studies presented in this thesis would have avoided the effect that similar subjects could have on orthostatic tolerance results, which was particularly important since there was no valid reason to exclude this type of subject from any of the studies in this thesis (3.1).

The lack of an increase in orthostatic tolerance following daily exposure to orthostatic stress has been discussed in detail in chapter 3, section 3.2.4. However, there

appears to be confusion regarding the effect of repeated orthostatic stress on orthostatic tolerance and the positive effects of repeated orthostatic stress on intolerance following head-down bed rest. Using lower body negative pressure (LBNP) as a countermeasure for the CV deterioration that occurs during bed rest (Miller et al, 1964), reductions in plasma volume and orthostatic tolerance have been attenuated (Stevens et al, 1966; Guell et al, 1991; Pavy-Le Traon et al, 1995). Yet, when daily exposure to orthostatic stress was administered in the second study in chapter 3 without bed rest, it did not improve orthostatic tolerance or alter indices for changes in plasma volume (see 3.2.4). This discrepancy may be related to the LBNP protocols used in these studies. In studies using orthostatic stress during bed rest, the LBNP protocols did not induce pre-syncope and were applied for up to one hour per day (e.g. Stevens et al, 1966). Therefore, it may be possible to induce improvements in orthostatic tolerance by application of a prolonged, single intensity orthostatic stress on a daily basis. Furthermore, the phenomenon of pre-syncope itself or the events following test termination (e.g. possible central hypervolaemia) may have abolished the positive influence of the orthostatic stress prior to pre-syncope in the study detailed in chapter 3, section 3.2, although this suggestion is speculative.

One possible mechanism for this suggestion could be related to a perfusion-reperfusion disturbance to the kidney immediately following test termination, thus affecting the kidney's handling of Na^+ and water (see 3.2.4). However, it is also important to consider that when head-down bed rest was combined with exposure to orthostatic stress, orthostatic tolerance was simply maintained, during conditions that were likely to have resulted in a reduction in CV control of BP homeostasis (Stevens

et al, 1966; Guell et al, 1991; Pavy-Le Traon et al, 1995). Therefore, the positive effects of applying orthostatic stress may only become apparent when the human CV system is subjected to stresses that would otherwise result in a deterioration of CV control of BP homeostasis (i.e. head-down bed rest).

6.2 Human CV control of blood pressure homeostasis and problems regarding the expression of BP changes during orthostatic stress.

As stated in chapter 3 (see 3.1.4), there are difficulties associated with analysing BP data recorded during pre-syncope orthostatic stress tests, since there was a wide variation in orthostatic tolerance between subjects in the present studies. The methods for BP data analysis used in the presented studies have included; expressing BP responses relative to orthostatic tolerance, changes from control to the last recording prior to pre-syncope and the time course changes up to the commonly tolerated magnitude of orthostatic stress. None of these methods are without limitations. For example, reporting BP changes relative to tolerance to orthostatic stress revealed no differences in the BP responses despite differences in tolerance (see 3.1). Furthermore, reporting the total change in BP does not offer insight into possible differences in the time course changes during the orthostatic stress leading to pre-syncope. Conversely, presenting time course changes in BP up to the commonly tolerated magnitude of orthostatic stress compares data when subjects are exposed to differing levels of orthostatic stress relative to their tolerance (see 3.1.4). A possible solution to this would be to report both time course changes up to the commonly tolerated orthostatic stress and the magnitude of changes from control to the last recording prior to pre-syncope (as in 5.2 for HR and SV).

The lack of a difference in BP responses at relative intensities of orthostatic stress was perhaps not surprising since in chapter 4 increases in orthostatic tolerance following isometric leg training were not accompanied by a different magnitude of change in BP during orthostatic stress. Furthermore, BP changes up to pre-syncope have been similar regardless of whether orthostatic tolerance changes or not (chapters 3 and 4). Previous studies have also reported no differences in the responses of mean arterial pressure (MAP), when orthostatic tolerance increased following repeated exposure to LBNP (Lightfoot et al, 1989) or showed no change following repeated exposure to LBNP (Lightfoot et al, 1991). One possible solution to investigating BP responses to orthostatic stress following changes in tolerance would be to report the slope of SBP reductions from control to immediately prior pre-syncope. From the present (see 3.1 and 4) and previous (Lightfoot et al, 1989; Lightfoot et al, 1991) data it would appear that increases in orthostatic tolerance are accompanied by attenuated responses in BP since the change in BP was the same, even though subjects tolerated orthostatic stress for longer periods of time. These responses in BP during orthostatic stress do not appear to be affected when resting BP was reduced following isometric training of the arms. However, resting BP was not reduced immediately prior to the post training test and this may be a critical factor in examining the effect of changes in resting BP on the control of BP homeostasis to orthostatic stress (see chapter 4).

Attenuated reductions in MAP have been shown previously following limitations in lower limb blood pooling during non-presyncopal orthostatic stress by either increased lower limb muscle tension or use of anti-shock trousers (Smith et al, 1987; Halliwill et al, 1998 respectively). However, whilst these altered BP responses to

orthostatic stress may indicate an increase in tolerance, orthostatic tolerance was not assessed in these studies and therefore the effect of the altered BP responses to orthostatic stress on tolerance remains uncertain.

6.3 Responses of the heart during orthostatic stress, in human CV control of blood pressure homeostasis.

Blood pressure is principally regulated by changes in \dot{Q} (SV and HR) and peripheral vascular resistance (FBF and CC changes). During orthostatic stress, \dot{Q} and SV have been shown to reduce, despite increases in HR (Stevens, 1966; Blomqvist and Stone, 1983; Al-Shamma and Hainsworth, 1985), which do not entirely compensate for the reduction in SV (Rowell, 1993). Studies in this thesis have demonstrated increases in HR during orthostatic stress and in chapter 5 it was clear that the increase in HR was not sufficient to maintain \dot{Q} as SV decreased. Measures of FBF were also made during orthostatic stress (see 5.2) and showed reductions (peripheral vasoconstriction), which were consistent with previous studies (Stevens et al, 1966; Johnson et al, 1974). Therefore, changes in these components of human CV control of blood pressure can be used to indicate CV responses to disturbances in BP homeostasis during orthostatic stress.

The inadequacy of HR responses to orthostatic stress in terms of maintaining \dot{Q} , are known to be related to the acute reduction in ventricular filling pressure at the onset of orthostatic stress (Rowell, 1993). Therefore, under conditions of improved venous return and ventricular filling pressure, a HR response of greater magnitude would

perhaps be expected, resulting in improved maintenance of \dot{Q} . However, increases in orthostatic tolerance (chapters 3, 4 and 5) were not accompanied by more rapid responses in HR during early orthostatic stress. After three repeated exposures to orthostatic stress (see figure 3.3), increased tolerance was not explained by differences in the HR responses. However, since subjects in the third test were more tolerant, there may have been a delay in the time to peak HR. Indeed, following isometric exercise training of the legs and during hypercapnic acidosis, increases in the time to peak HR during orthostatic stress accompanied the increased orthostatic tolerance and there was a significant relationship between changes in these two variables (figure 4.4). Therefore, HR responses to orthostatic stress were perhaps dependant on the magnitude of central blood volume shifts and the degree of blood pooling in the capacitance vessels of the lower limbs, which in part determine changes in central venous pressure (CVP).

However, the delay in time to peak HR observed with concomitant increases in orthostatic tolerance (chapters 3, 4 and 5), suggests that the HR response during early orthostatic stress may prove to be a reliable predictor of within subject changes in orthostatic tolerance without inducing pre-syncope. Nevertheless, it is important to note that changes in the time to peak HR must be considered in relation to the peak HR recorded during tests. In chapter 5 (5.2) there was an increase in the time to peak HR and an increase in orthostatic tolerance. However, in addition there was a significant increase in peak HR and a further 22.3% reduction in SV immediately prior to pre-syncope under hypercapnic conditions. Therefore, during early orthostatic stress there was not a difference in the HR responses between hypercapnia and

normocapnia (figure 5.1), thus necessitating assessment of orthostatic tolerance in order to adequately explore differing responses between these two conditions.

The role of the carotid sinus baroreceptors in the human CV responses to orthostatic stress appear to be limited since there were no significant changes in R-R interval or FBF during carotid sinus baroreceptor unloading. However, the effectiveness of applying external pressure to the neck may not induce similar changes in carotid sinus distending pressure that are likely to occur during orthostatic stress.

6.4 Disturbances in total and/or central blood volume in relation to tolerance to orthostatic stress.

An important consideration when investigating tolerance to orthostatic stress is the volume of blood (or its plasma component) and its distribution within the human CV system. Previously, it has been demonstrated that changes in plasma volume are related to changes in orthostatic tolerance (Luft et al, 1976; El-Sayed and Hainsworth, 1996; Mtinangi and Hainsworth, 1998). Importantly, during orthostatic stress there is a known loss of intravascular plasma volume into interstitial spaces induced by the increase in intravascular hydrostatic pressure, and thus capillary transmural pressure, in the dependant limbs, via the Starling-Landis principle (Fawcett and Wynn, 1960; Hagan et al, 1978; Noddeland et al, 1981; Lundvall and Bjerkhoel, 1995). However, despite repeated exposure to orthostatic stress (chapter 3), and presumably increases in transcapillary filtration, there were no differences in the indices used for changes in plasma volume (see chapter 2) and therefore this did not explain the differing results of the studies in chapter 3 when increases in

orthostatic tolerance were shown following three repeated tests, but no change in tolerance following five repeated tests.

However, it would appear from these (chapter 3) and previous (Lightfoot et al, 1989; Lightfoot et al, 1989) data that orthostatic tolerance is highly reproducible, except for a small core of subjects who appear to develop an adaptation to orthostatic stress (see 3.1), that is at present unknown, and that these peculiar subject characteristics were not present in the second repeated orthostatic stress exposure study (see 3.2). In study 3.2 where subjects were exposed to orthostatic stress to pre-syncope daily for five days, there were no changes in tolerance or fractional excretion of Na^+ . This suggests that any reductions in plasma volume that occur during orthostatic stress are restored within 24 hours of the test either through re-absorption of capillary filtrate from the interstitium or increased retention of Na^+ and therefore water by the kidney.

Reductions in Na^+ excretion have been demonstrated previously following orthostatic stress via the Henry-Gauer reflex (Gilbert et al, 1966; Mauran et al, 1998; see 3.2.4). Capillary filtration during orthostatic stress is abolished when tissue fluid pressure and capillary hydrostatic pressures equilibrate but this does not occur until after an approximate movement of 600ml of plasma volume into the interstitium (Hagan et al, 1978). The established pooling of blood in the lower limbs during orthostatic stress compounds this reduction in effective central blood volume. Upright posture results in an increase in lower limb venous volume approaching 500mL (Gauer and Thron, 1965) with an additional 200 - 300mL pooled in the pelvic area (Wolthuis et al, 1974). However, upright posture is approximately equivalent to -50mmHg of LBNP (Wolthuis et al, 1974) and the majority of the subjects in the present studies tolerated

much higher levels of negative pressure (up to -100mmHg), and therefore the total volume of pooled blood may have been much higher. Although the total percentage volume of pooled blood just prior to pre-syncope is at present unknown, it is clear that blood pooled in the lower limb and pelvic area capacitance vessels represents the most important disturbance to central blood volume during pre-syncopal orthostatic stress.

If the volume of blood pooled in the lower extremity during orthostatic stress could be limited, it seems likely that the control of BP homeostasis would improve. Reductions in orthostatic tolerance have been shown previously following endurance exercise training and this reduction was suggestive of an increase in lower limb vascular compliance (Stevens et al, 1992). Conversely, it has been suggested that increases in lower limb muscle tone or muscle mass are associated with increased orthostatic tolerance (Smith et al, 1987; Convertino et al, 1988, respectively). In the studies in chapter 4 and subsection 5.2, there was evidence to suggest that blood pooling in the lower limbs was either restricted by increases in leg volume (chapter 4) or attenuated by improved venous return, perhaps via the respiratory pump, indicated by the increased minute ventilation and an attenuated increase in calf circumference (see 5.2). Further evidence in support for this speculation regarding the volume of pooled blood in the lower limbs during these studies was shown by the increase in orthostatic tolerance and a delay in the time to peak HR.

However, following isometric exercise training of the legs (chapter 4) the increase in time to peak HR was not accompanied by an increase in peak HR during orthostatic stress. Conversely, hypercapnia (5.2) induced both an increase in peak HR and the

time to peak HR. It could be argued that an increase in the time to peak HR, but no increase in peak HR itself, suggests that the reduction in central venous pressure and thus the loss of central blood volume immediately prior pre-syncope was not different when compared to before isometric training of the legs. However, during hypercapnia, compared to normocapnia there was an increase in time to peak HR, peak HR and the reduction in SV was 22.3% greater immediately prior to pre-syncope. This suggests that the total reduction in central blood volume was greater before the onset of pre-syncope which may have been related to increases in cerebral blood flow during hypercapnia (Rowell, 1986; Serrador et al, 2000).

However, a delay in the onset of pre-syncope during increasing reductions in central blood volume may not automatically propose improved regulation of BP homeostasis in the same way as the isometric leg training data suggests. During orthostasis the occurrence of syncope could be interpreted as a CV response to the inability of the CV system to correct the disturbance in central blood volume and distribution. At this point the only possible solution would be to adopt the supine position and syncope will result in the rapid assumption of this posture. Syncope during orthostasis could be regarded as a protective response to prevent the reductions in central blood volume from exceeding a critical level. Possibly, from the orthostatic tolerance of the subjects in 5.2 during normocapnia, a given reduction in central blood volume (indicated by the reductions in SV) was required before the onset of pre-syncope. However, during hypercapnia the reductions in SV indicated further reductions in central blood volume before the onset of pre-syncope, suggesting that the possible protective element of pre-syncope was compromised.

6.5 Proposed future studies.

Further examination of the differences between subjects that show increases in orthostatic tolerance following repeated exposure to orthostatic stress and those who do not, may improve the general understanding of human CV responses to orthostatic stress. In addition, exposure to orthostatic stress at time intervals of less than 24 hours may provide further evidence concerning the reproducibility in human CV responses to and following orthostatic stress, including renal handling of Na^+ excretion. Moreover, monitoring the time course changes in fractional excretion of Na^+ during the 24 hours following exposure to pre-syncope may provide important information regarding the role of the kidney in the recovery of plasma volume following orthostatic stress.

Isometric exercise training has been shown to be an effective method by which resting BP can be reduced. However, in order to assess the effect of such a reduction in resting BP on orthostatic tolerance, the timing of the post exercise training orthostatic stress test appears critical, since the reductions in resting BP are apparently transient. Therefore, it may be necessary to test subjects' orthostatic tolerance 24 hours after the completion of isometric exercise training. Other methods for reducing BP include administration of diuretics or β - adrenoreceptor antagonists. However, these agents may effect human CV responses to orthostatic stress themselves by either reducing blood volume or affecting responses of the heart. Conversely, an angiotensin converting enzyme (ACE) inhibitor would induce a reduction in peripheral resistance, without effecting human CV reflexes (Neal, 1997).

This may provide improved conditions for examining the importance of resting BP on tolerance to orthostatic stress.

Assessment of human CV receptor control of peripheral blood flow provides important information regarding tolerance to orthostatic stress. The effectiveness of applying external pressure to carotid sinus regions in unloading the carotid sinus bareceptors should be questioned further and perhaps new, more effective methods could be developed to examine the role of carotid sinus baroreceptors in the human CV responses to orthostatic stress. Furthermore, the role of the carotid sinus baroreceptors in the human CV responses to orthostatic stress could potentially be assessed by applying progressive neck suction with simultaneous orthostatic stress, thus preventing carotid sinus baroreceptor unloading under these conditions.

In 5.2 there was a significant increase in orthostatic tolerance during chemoreceptor stimulation (hypercapnia). However, during hypercapnia there were substantial increases in minute ventilation (V_E) and the respiratory pump is known to play an important role in the return of blood to the right heart. Increased effectiveness of the respiratory pump may impact on human CV responses to orthstatic stress. However, by standardising V_E and using a computerised gas mixing system to induce hypercapnia (Shoemaker et al, 2001), the influence of these large changes in V_E on human CV responses to orthostatic stress during hypercapnia can be removed.

6.6 General conclusions.

Studies in this thesis have examined human CV control in relation to tolerance to orthostatic stress. The evidence presented has provided improvements in the understanding of the effects of repeated exposure to orthostatic stress, the effectiveness of isometric exercise training in reducing resting blood pressure and assessing subsequent effects on orthostatic tolerance, and the effects of baroreceptor and chemoreceptor stimulation on responses to orthostatic stress.

In the preceding discussion these studies have shown that human CV responses and tolerance to orthostatic stress appears to be reproducible when tests are repeated. However, possible increases in tolerance to orthostatic stress may present in a small core of subjects and this must be accounted for in future study designs. Any special characteristics that these subjects might have, that may predispose them to increasing orthostatic tolerance upon repeated exposure to orthostatic stress, is at present unknown,

There are difficulties in expressing blood pressure responses to orthostatic stress. In order to provide evidence for differences in BP responses to orthostatic stress when tolerance has changed, BP recordings must be expressed as time course changes up to pre-syncope. Since between subject variability in orthostatic tolerance is high, a possible solution would be to report both BP responses to a commonly tolerated negative pressure and the magnitude of change from control to immediately prior to pre-syncope. However, it is reasonable to suggest, based on evidence provided in this thesis, that the magnitude of SBP reduction prior to pre-syncope was not

different with changes in orthostatic tolerance, but the rate at which it changes may be different.

Changes in the heart rate (HR) responses to orthostatic stress may indicate changes in orthostatic tolerance. However, consideration must be given for possible changes in peak HR prior to pre-syncope, since these changes may effect the pattern of HR responses during early orthostatic stress.

Changes in the volume of blood pooled in the lower limbs and thus the degree of central hypovolaemia during orthostatic stress, may affect orthostatic tolerance. However, when changes in the volume of blood pooled in the lower limbs is accompanied by possible changes in cerebral blood flow, for example during hypercapnia, reduction in central blood volume below a level at which pre-syncope would normally present, may occur.

Chapter 7.

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